Heterogeneity between the hepatic artery and the portal vein in the isolated perfused rat liver in situ

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HETEROGENEITY BETWEEN THE HEPATIC ARTERY AND THE PORTAL VEIN IN THE ISOLATED PERFUSED RAT LIVER IN SITU

submitted by

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This research has been carried out in the School of Pharmacy and Pharmacology, University of Bath, under the supervision of Dr. P.N. Bennett M.D., F.R.C.P and Dr. L.J. Notarianni Ph.D.

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DEDICATION

I wish to dedicate this thesis to my family who have have given me endless support throughout the period of my research.

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SUMMARY

The present study investigated heterogeneity between the hepatic artery and the portal vein in the isolated perfused rat liver in situ. The extraction ratio of lignocaine was found to increase as portal vein flow contribution was raised under conditions of constant total liver blood flow. This change in the extraction ratio of the drug was also found to be related to the perfusion pressure in the hepatic artery. During increases in hepatic artery perfusion pressure the extraction ratio of lignocaine was reduced under conditions of constant HA:PV flow ratio. The reduction in extraction ratio of lignocaine during changes in the HA:PV flow ratio are possibly due to mechanical changes induced by increases in hepatic artery perfusion pressure rather than a reflection of heterogeneous metabolic channels.

The metabolic profile of lignocaine was also found to alter during changes in HA:PV flow ratio. As the portal vein flow contribution increases so the recovery of 3-hydroxylignocaine is reduced and the recovery of deethylated metabolites increased. This effect was not caused by changes in perfusion pressure and reflects a degree of metabolic environment heterogeneity between the two blood supplies.

Both the hepatic artery and the portal vein were found to possess α and β-adrenoreceptors. A degree of heterogeneity exists between the β-adrenoreceptor population of the hepatic artery and the portal vein. The hepatic artery has a predominance of β_1-adrenoreceptors and the portal vein a predominance of β_2-adrenoreceptors.

Studies using hydralazine in the rat showed that the drug has an effect on the rate of elimination of lignocaine and that this is probably due to the effect of active hydralazine metabolites on the systemic circulation.

CHAPTER 1

INTRODUCTION

1) LIVER ANATOMY.

(a) Gross Structure.

The liver has two main blood supplies, the hepatic artery and the portal vein. The hepatic artery forms from the coeliac axis of the aorta. In the rat this gives rise to the common hepatic artery which divides into the left and right hepatic arteries, the right gastric artery and the gastroduodenal arteries. The portal vein stems from the confluence of the splenic, inferior mesenteric and superior mesenteric veins. Blood drains from the liver via three hepatic veins which are short and connect to the inferior vena cava. In the livers' of cats, dogs and man the hepatic artery supplies 25% to 30% of total liver blood flow, (Greenway, 1971). In rats this ratio may be lower, 19%, in normal tissue (Conway et al.,1984). However, it would seem that the artery may supply 65% of the total liver oxygen requirement (Greenway, 1971). The liver mass may be divided into the left and right major lobes; each may be further divided into a main and accessory lobe.

(b) Fine Structure.

The hepatic artery and the portal vein enter the liver mass surrounded by a sheath of connective tissue known as Glissons capsule. Bile and lymph ducts also run within this capsule. These sheathed bundles, or portal triads, divide frequently, eventually giving rise to terminal branches which pass through the connective tissue sheath. Terminal branches of both the hepatic artery and the portal vein drain into the sinusoids. These sinusoids are the connecting channels between the two input vessels and the hepatic veins.

The sinusoids are numerous within the liver and run between folds of parenchymal cells only one or two cells thick. Measurements of sinusoid dimensions have yielded a wide variation in values depending on the preparation used. Rappaport (1973), reported the diameter of the sinusoids as 7-15um, Grisham et al. (1975), 20-40um and Koo et al. (1979c, 1979d, 1979e), 6.2-6.4um. The results of studies to show the length of sinusoids also varies. Rappaport (1973), measured their length at 250um, Koo et al. (1979c, 1979d), found sinusoids to be 18-27um long. Rappaport (1981), studied human sinusoids and reported their length to be 225-477um. As well as showing

a degree of variation in size sinusoids also cannot be considered to be a simple series of parallel channels between inflow and outflow. Brauer (1963), reported the presence of direct portal-venous sinsoids and also that smaller sinusoids exist which run at right angles to the general direction of flow. McCuskey (1966), reported these sinusoids to be intersinusoidal connections.

The walls of the sinusoids are composed of two types of cells, the epithelial cells and the Kupffer cells. The vast majority of cells present in the sinusoid walls are epithelial cells. These are flat and thin with an irregular circumference, (Jones et al., 1977). The Kupffer cells are thought to be reticuloendothelial in nature because of their ability to ingest dyes, (Ham, 1974). Kupffer cells are stellate in appearance with ruffles on their surface (Jones et al., 1977).

The epithelial cells of the liver show two characteristics which allow intimate contact between fluid flowing in the sinusoids and the surrounding parenchymal cells. Firstly they possess fenestrations, (Novikoff, 1959; Burkel, 1966; Jones et al., 1977). These are holes in the epithelial cells which allow the passage of plasma out of the sinusoid lumen

but not large protein molecules and blood cells. They may be classified into two types, large fenestrations between 1.0-3.0um in diameter and smaller fenestrations of 0.1um diameter (Grisham et al., 1975; Wisse et al., 1983). Fenestrations are not found in the Kupffer cells (Jones et al., 1977). Secondly, for much of its length the sinusoid lacks a basement membrane. Burkel (1966), showed that there are three distinct regions along the length of a sinusoid. Where the portal vein and the hepatic artery join the sinusoid, epithelial cells possess a basement membrane and lack fenestrations. In the middle portion of the sinusoid, which forms about 90% of the total sinusoid length, the epithelial cells have no basement membrane and have numerous fenestrations. Where the sinusoid connects to the venous outflow, the epithelial cells loose their fenestrations and gain a basement membrane. For the majority of the sinusoids length, the lack of a basement membrane and the appearance of fenestrations permit intimate contact between plasma and the liver parenchymal cells.

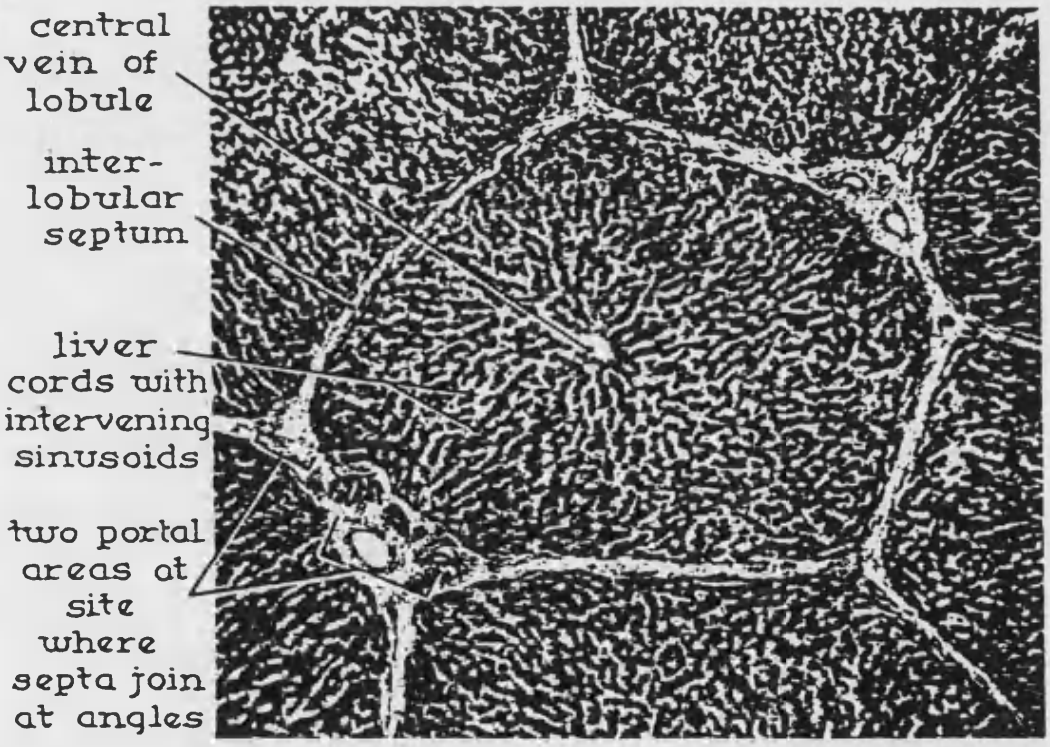
Surrounding the epithelial cells are the parenchymal cells of the liver. These usually form cords which are two cells thick. Between these cords run bile canaliculi. Thus, parenchymal cells have one

side exposed to the sinusoidal environment whilst the opposite side faces the bile canaliculus. The sinusoidal surface of the parenchymal cells is most important in the uptake of molecules into the cell and the surface of this side of the cell is rich in microvilli. In the rat these microvilli are 0.5um long and 0.1um in diameter, with 25-50 microvilli per um^2 cell surface area, (Grisham et al., 1975). The microvilli protrude into the gap between the parenchymal cells and the epithelial cells. This area is known as the space of Disse. It is filled with plasma but devoid of blood cells. The parenchymal microvilli increase the surface area of the cell in contact with the fluid in the space of Disse. This increases the uptake area of the cell and improves the efficiency of the removal process from the blood. Parenchymal cells are also rich in mitochondria (Loud, 1968) and endoplasmic reticulum, (Jones et al., 1977); two essential cell components required for metabolism.

The passage of blood through the sinusoidal system of the liver affords an excellent environment for metabolism. There is intimate contact between the blood and the metabolising parenchymal cells. These are well equipped to carry out both active transport and endoplasmic chemical reactions.

Figure 1.2.1

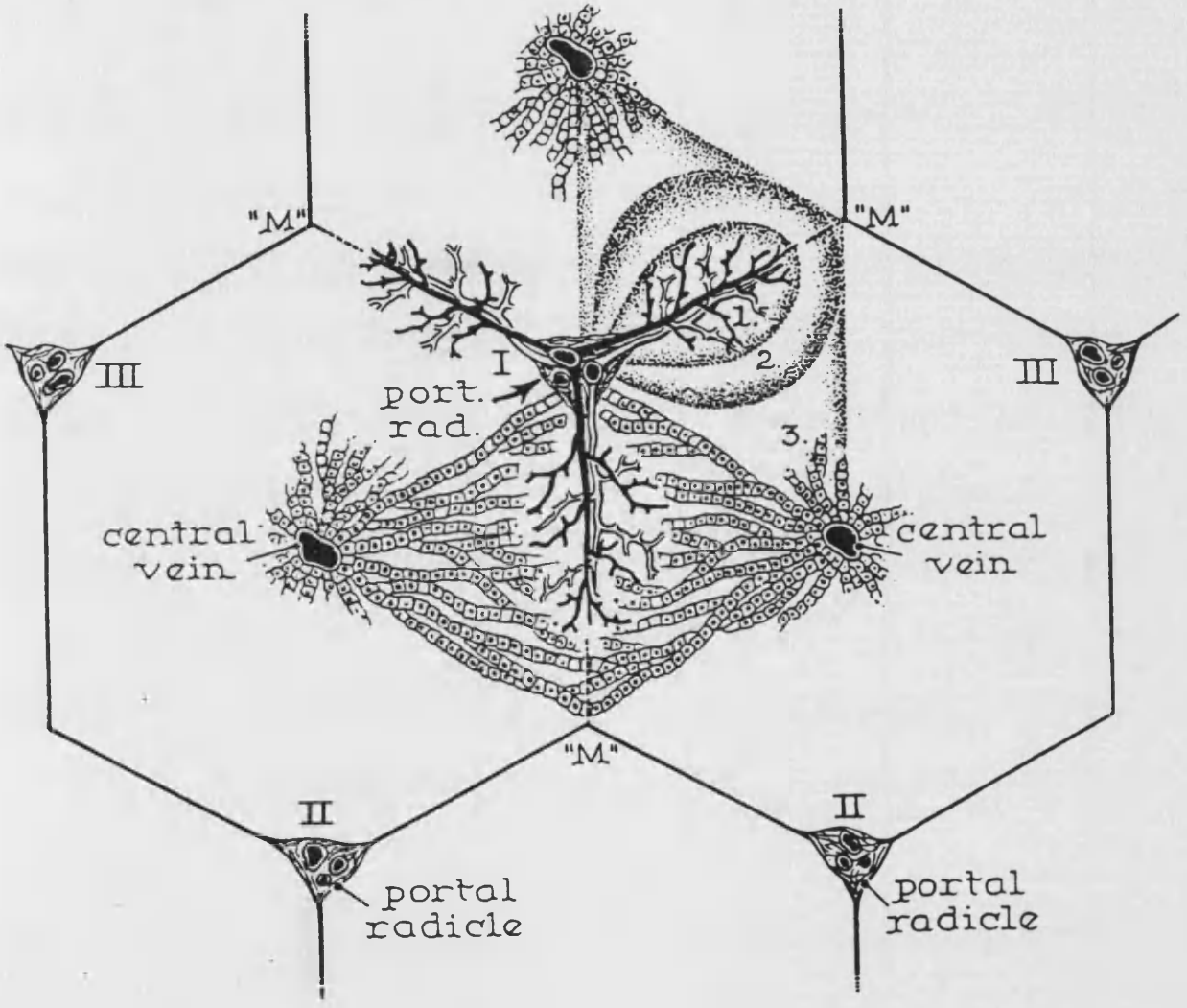
A Low Power Photomicrograph of an H and E Section from the Liver of the Pig, Illustrating the Classic Hexagonal Lobule



Taken from Ham (1974).

Figure 1.2.2

A Diagram to Illustrate the Acinus Relation to the Classic Hexagonal lobule



"1" = Acinus Zone 1 or the Periportal region.

"2" = Acinus Zone 2 or the Midzonal region.

"3" = Acinus Zone 3 or the Centrilobular region.

Taken from Ham (1974).

2) LIVER VASCULATURE.

(a) The Acinus.

The terminal branches of the hepatic artery and the portal vein are arranged in an approximately hexagonal form around a central vein. This is most clearly seen in the liver of pigs, (fig 1.2.1). However, these hepatic hexagonal lobules are not the functional units of the liver. Rappaport (1958), identified the functional unit of the liver as the acinus which he defined as the parenchymal mass that surrounds the axis of a terminal hepatic arteriole and the portal venule growing out of similar pre-terminal branches. Since the terminal vascular branches follow an hexagonal pattern and since blood from these will drain into central veins on either side of the terminal axis the acinus has a roughly diamond shape, (fig. 1.2.2).

Actual anatomical evidence for these units has been shown by Jones et al. (1977). Rappaport (1981), measured the maximum dimensions of human liver acini and found them to be 1113-1908 um in length, 398-1113 um in thickness and 636-1590 um in width, with an average volume of 1.26 mm^2. It was also postulated by Rappaport (1958), that the

sinusoids closest to the terminal axes should contain blood of higher oxygen content than those nearer to the central vein. Miller J.A. et al. (1977), showed an oxygen gradient did indeed exist between blood in the sinusoids close to terminal branches and those sinusoids nearest to the central vein. This has led to the division of the acinus into three zones, (Rappaport, 1980). Zone 1 or the periportal zone, consists of those sinusoids immediately surrounding the terminal branches of the portal vein and hepatic artery. This zone possesses blood which is rich in oxygen. Zone 3 or the centrilobular zone, consists of those sinusoids surrounding the central vein which contain blood of a lower oxygen concentration. Zone 2 or the midlobular zone, consists of those sinusoids between zones 1 and 3 possessing an intermediate oxygen concentration, (fig. 1.2.2).

(b) Intrahepatic connections.

The concept of the liver acinus being the functional unit of the liver has now been widely accepted. However, there still remains controversy over the arrangement of the terminal portal venule and hepatic arteriole connections with the sinusoids. Elias et al. (1953), showed the presence of arterial

connections to thoroughfare artery channels which transverse the three acinar zones without mixing with portal blood. Observations by McCuskey (1966) in rats showed that portal venules commonly gave rise to sinusoids directly. However, connections between the hepatic artery and these sinusoids suggested that some sinusoids were perfused intermittently by arterial blood alone. He also observed direct arterio-portal anastomoses prior to the blood entering the sinusoids. He noted that the Kupffer cells often acted as sphincters and formed inlet and outlet controls for the sinusoids. Bloch (1955), had previously observed these sphincters and later confirmed that they were Kupffer cells, (Bloch, 1970).

Burkel (1970), reported the presence of direct arteriole connections between the intralobular and terminal distributing veins, however, these connections are rare and the majority of arterioles join to sinusoids directly or in conjunction with portal venule connections. Experiments by Grisham et al. (1981), using hepatic casts of microvessels showed that the majority of hepatic arteriole connections were supplied by the hepatic biliary plexus. This is a complex of capillaries which surrounds the portal tracts. Nopanitaya et al. (1978); Ohtani et al. (1978)

and Grisham et al. (1981), observed direct connections from arterioles to portal venules and direct arteriole connections to both zone 1 and zone 3 of the acinus; the latter being extremely rare. These findings are in contrast to those of Hase et al. (1966), who reported no such arteriole connections with zone 3. The peribiliary plexus mainly supplies the sinusoids in zone 1 but it also has connections with the portal venules. These connections between both the hepatic artery and the peribiliary plexus with the portal vein confirm observations made by Geumei (1969), who showed unidirectional arterio-portal shunts. Grisham et al. (1981), provided evidence that the portal vein supplies direct connections only to zone 1 of the acinus.

Efferent branches of the peribiliary plexus form an important source of blood for many sinusoids in those parts of the liver where the distributing portal venules are poorly developed, (Ohtani, 1978 et al. and Nopanitaya et al., 1978). The peribiliary plexus sinusoid connections are especially important in the perihillar regions of the liver, (Grisham et al., 1981).

It would seem from these observations that the majority of the sinusoids are perfused by

both hepatic artery and portal venous blood but that some sinusoids may be perfused by portal or arterial blood alone. Also, although sinusoids may be perfused by a mixture of both hepatic artery and portal vein blood, the proportions of each supply may not be the same for each sinusoid. A problem that arises with the theory that sinusoids are perfused by a mixture of arterial and portal blood is how the portal vein blood under pressures of 3-4 mmHg flows against an hepatic artery pressure of 30-35 mmHg, (Rappaport, 1981). The most likely answer involves a reduction in arteriole pressure through the peribiliary plexus and the control of arteriole blood by sphincters which intermittently allow jets of arteriole blood to pass into sinusoids. Seneviratne (1949), Elias et al.(1953) and Rappaport (1981), have observed pulsating blood flow in the sinusoids which may be a result of intermittent flow.

Experimental observations have shown the existence of direct hepatic artery connections to sinusoids and also indirect connections via the peribiliary plexus. The complexity of the connections provides the scope for sinusoids to be perfused to varying degrees by the hepatic artery or portal vein.

3) ACINAR HETEROGENEITY.

As well as showing a degree of heterogeneity in the way in which blood supplies connect to the sinusoids in the various acinar zones, the three acinar zones also exhibit zone dependent characteristics. These have been grouped under two subheadings: structural heterogeneity, (describing the structural differences) and biochemical heterogeneity, (describing the distribution of the enzymes and cofactors between the zones).

(a) Structural Heterogeneity.

Hase et al. (1966), found in rats, that the sinusoids of acinar zone 1 are more anastomatic whereas the sinusoids of zone 3 tend to be straighter with fewer interconnections. Koo et al. (1975), showed that the blood flow within sinusoids differs. Some sinusoids connect the portal vein directly to the hepatic central vein; these have a fast flow. Branching sinusoids have a medium flow and interconnecting sinusoids a slow flow rate. Because there are more interconnecting sinusoids in zone 1, (Hase et al., 1966) these flow velocities may be zone dependent. Wisse et al. (1983), showed that the sinusoids of zone 1 were more tortuous.

Miller D.L. et al. (1979, 1981), showed that the internal surface area to volume ratio of sinusoids is larger in zone 1. These observations were confirmed by Wisse et al. (1983). The significance of the surface area to volume ratio is that the higher the ratio the more likely a given molecule will pass through the epithelial cell fenestrations and collide with the surface membrane of a parenchymal cell. This process is essential for metabolism and the rate at which solute makes contact with the cell wall affects the rate of metabolism.

Loud (1968), showed that the parenchymal cells of zones 1 and 3 were similar in size. However, he did show that the mitochondria in cells of zone 3 occupied less of the total cell volume and although smaller they were more numerous in this zone. He showed that rough endoplasmic reticulum volume was similar in all zones but that the volume of smooth endoplasmic reticulum was higher in the cells of zone 3. The significance of these findings may be too small to produce any zonal differences in metabolism as 80% of the liver parenchymal cells did not display these differences. It was only those cells at the extremes of the acinus which exhibited such heterogeneity.

Wisse et al. (1983), showed that the fenestrations observed in zone 1 were on average larger than those of zone 3. Grisham et al. (1975), found that the epithelial cells of zone 1 possessed more of the larger type of fenestrations. The epithelial cells of zone 1 therefore have a greater porosity. The overall result is that solute molecules have a greater chance of colliding with liver parenchymal cells in zone 1. In theory the rate of removal of solute would be higher in zone 1 than zone 3. The structural heterogeneity within the acinus tends to favour a higher uptake and metabolism in zone 1 but the acinus also shows a degree of biochemical heterogeneity with respect to enzyme distribution.

(b) Biochemical Heterogeneity.

Shank et al. (1959), showed that in rats glucose-6-phosphate dehydrogenase, phosphoglucoisomerase isomerase, lactate dehydrogenase and alkaline phosphatase activities were all higher in zone 1 than zone 3. Isocitrate dehydrogenase and glutamate dehydrogenase levels were lower in zone 1 than zone 3. Katz et al. (1976), demonstrated that glycolosis and gluconeogenesis may display some zonal heterogeneity. Later Katz et al. (1977), also fructose 1,6, biphosphatase levels to be

higher in zone 1 but that glucokinase activity was lower in zone 1. Guder et al. (1976), found more pyruvate kinase but less phosphenol pyruvate carboxykinase activity in zone 3. Wimmer et al. (1979), found higher levels of succinate dehydrogenase, malate dehydrogenase and lactate dehydrogenase in zone 1 but greater isocitrate dehydrogenase and glutamate dehydrogenase activity in zone 3. These observed zonal differences have been taken to indicate that zone 1 is predominantly involved in gluconeogenesis while cells of zone 3 participate in glycolosis. Gumucio et al. (1981), pointed out that the heterogeneity shown by the cells of the acinus in respect to glycolosis or gluconeogenesis may reflect their position or the availability of co-factors in these zones rather than actual enzyme levels.

Recently attention has focused on the enzymes responsible for drug metabolism and their heterogeneity within the acinus. Gooding et al. (1978), showed that cytochrome P_{450} was generally concentrated in the centrilobular zone. Baron et al. (1978), looked at the different induceable forms of cytochrome P_{450}. He showed that the phenobarbital induced form of cytochrome P_{450} was concentrated in zone 3. However, the methylcholanthrene induced cytochrome P_{448}

was more evenly spread within the acinus. Baron et al. (1981), showed that the cytochrome P_{450} forms are also more active in the centrilobular region. He believes that this may be in part due to co-factor differences.

An important enzyme involved in drug oxidation is NADPH-cytochrome P_{450} reductase. Redick et al. (1980) and Ji et al. (1980), showed the levels of this enzyme were much lower in zone 1 than in zones 2 and 3. To measure actual enzyme activities within the three zones Ji et al. (1981) and Thurman et al. (1983) looked at the conversion of the non-fluorescent 7-ethoxycoumarin to the fluorescent 7-hyroxycoumarin by the mixed function oxidase systems. They found the enzyme activities to be higher in zone 3. James et al. (1981), using selective zone 1 and zone 3 damage showed that the mixed function oxidase systems were concentrated in zone 3.

Phase II metabolism has also been studied. Pang et al. (1981a), using normal and retrograde perfusion in the rat, showed that sulphate conjugation was concentrated in zone 1. Pang et al. (1981b), using harmol as an indicator again showed that sulphate conjugation was concentrated in zone 1. and that the enzyme sulphotransferase has a high

affinity but low capacity. Pang et al. (1981), using harmol and Conway et al. (1982), using 7-ethoxy-coumarin showed that glucuronide conjugation was evenly distributed within the acinus. James et al. (1981), using the less sensitive method of selective zonal damage, showed a zone 2 and 3 localisation of glucuronyl transferase, a zone 2 localisation of glutathione transferase and a zone 1 and 2 localisation of N-acetyl transferase.

The process of uptake is also important for drug metabolism. This is an active process and Novikoff (1959), showed ATPase activity is higher in zone 1. Conway et al. (1982), found that all parenchymal cells can take up hydroxycoumarin at the same rate. Groothius et al. (1982), showed that bile salt uptake of individual cells is uniform throughout the acinus. However, what is more important is the relationship of these cells to the input of blood. As cells of zone 1 are normally exposed to the highest solute concentration, they may remove most of the drug before it reaches zone 2 and 3 and so it appears that uptake is highest in zone 1. If retrograde perfusion is performed (Conway et al., 1982; Groothius et al., 1982), then the concentration gradient is reversed and the cells of zone 3 can take up more drug from the blood

stream. It appears with these drug metabolism markers that each parenchymal cell is capable of removing the drug from the circulation. However, because of their position within the acinus, cells of zone 3 do not appear to be as active in uptake as zone 3 solute levels are normally lower.

Care must be taken when interpreting the results of data on acinar heterogeneity. It is often not possible to distinguish whether zonal differences reflect actual biochemical alterations between the cells or are a result of the cells position with respect to blood input.

4) HETEROGENEITY BETWEEN THE HEPATIC ARTERY AND PORTAL VEIN.

The previous sections have outlined evidence for acinus zonal heterogeneity of vascular connections and metabolism. The possibility also exists that drugs passing into the liver via the hepatic artery may be treated differently than if they entered by the portal vein. If this phenomenon is to occur two conditions must be met by the liver physiology. First, there must either be some separate channels for the passage of hepatic artery and portal vein blood or that some sinusoids are perfused by differing proportions of the two blood supplies. Secondly, those sinusoids taking a preponderance of hepatic arterial or portal venous blood must have different enzyme environments to those carrying fully mixed blood.

(a) Evidence for separate hepatic artery and portal vein channels through the liver.

Most investigations in this field have been confined to cats and dogs and involve the use of radioactive tracers. Rees et al. (1964), using Xenon 133 showed that the washout curves were different depending on whether the tracer was perfused into the

hepatic artery or the portal vein. Hollenberg et al. (1966), using radiolabelled Krypton in dogs showed that injections into the hepatic artery gave a lower calculated total liver blood flow than injections into the portal vein. He concluded that separate channels must exist. Birtch et al. (1967), also using Krypton in dogs, divided the washout curves into three different decay rates. He found marked differences in the parameters obtained for the hepatic artery than for the portal vein. He also measured the parameters from a combined hepatic artery and portal vein injection. Thus, if the hepatic channels for each supply were totally separated then the sum of the hepatic artery and portal vein parameters should equal those found for the combined injection. This was not the case. Also, if the blood was totally mixed prior to passage through the liver there would be no difference between the hepatic artery and portal vein parameters. This also was not true. The conclusion must be that both separate and mixed routes exist.

Blumgart et al. (1977), carried out experiments in dogs in which he found that extrahepatic shunts exist between the gastroduodenal artery and the portal vein. When these are ligated, the portal vein and hepatic artery washout times for radioactive trac-

ers are identical. In the experiments of Rees et al. (1964), a gastroduodenal cannula was used to perfuse the hepatic artery and these extrahepatic shunts could account for the differences in transit times reported. However, in the experiments of Birtch et al. (1967), Krypton was injected into a cannula placed directly in the hepatic artery with the gastroduodenal artery ligated. In the experiments carried out by Hollenberg et al. (1966), a cannula was placed in the coeliac artery between the branching of the gastroduodenal artery and the liver. Therefore in the experiments by Birtch et al. (1967) and Hollenberg et al. (1966), shunts are unlikely to explain any differences in transit time.

Another possible explanation for the results obtained by Blumgart et al. (1977), is that ligation of the shunts prevented arterial blood from passing to the portal vein. This would result in an increase in hepatic artery flow and a decrease in portal vein flow. Thus, under ligated conditions the change in blood flow through each route may alter tracer transit times so that they become route independent. In the experiments of Blumgart et al. (1977), no measurements of flow are presented and so this remains purely conjecture.

Work by Lifson et al. (1970) and Griffen et al. (1970), showed that some areas of the liver were more highly perfused than others. However, these areas were more highly perfused by both the hepatic artery and portal vein.

(b) Evidence for separate metabolising environments.

Experiments have been carried out to examine variations in the space of Disse between the two blood supplies. This normally involves using radiolabelled red blood cells and radiolabelled albumin. The transit time of the albumin is slower through the liver as it is able to pass into the space of Disse, whereas red blood cells cannot. The difference in transit time reflects the size or accessibility of the space of Disse. Experiments in dogs by Cohn et al. (1969), showed that these transit times were not route dependent. Work carried out by Ahmad (1982), in the perfused rat, showed that the apparent space of Disse was larger when livers were perfused by the hepatic artery than the portal vein. This seems to indicate a difference in the space of Disse between the two routes.

Lautt et al. (1984), used the dye indo-

cyanine green as an indicator of liver uptake and and showed the process was not route dependent. However this work was carried out in cats where the extraction ratio of indocyanine green is only 25% and so may not represent the best marker of metabolism.

Experiments by Ahmad et al. (1981), have shown that the extraction ratio of lignocaine is dependent on the hepatic artery to portal vein flow ratio under conditions of constant total blood flow. This provides evidence for possible differences in enzyme environments between the two routes.

5) VASCULATURE RESPONSES WITHIN THE LIVER.

There is thus speculative evidence pointing to the existence of separate channels for the passage of arterial and portal blood through the liver and that these channels may metabolise drugs differently. If the route by which a drug takes into the liver is to have any physiological significance, there must exist the possibility that the blood flow through each route may be altered to vary the hepatic artery portal vein flow ratio.

(a) Methods of study.

The study of changes in vascular resistance in the beds of the hepatic artery and portal vein has been in the main confined to large animals such as cats and dogs where surgery is easier. Four preparations are commonly used to study the vascular beds of the liver.

i) The isolated perfused system.

In this preparation the liver is isolated from the general circulation and perfusion takes place using media with carefully controlled constituents. The preparations are also denervated as the rest of the animal is deprived of blood and therefore of oxygen.

ii) Long circuited preparations.

These preparations require careful surgery. They involve the cannulation of the hepatic artery and the transfer of femoral artery output via a second cannula to the hepatic artery. External flow meters and pressure gauges may be attached to this external connection The portal vein is also cannulated and perfused with the blood obtained from the mesenteric veins. An alternative method developed by Lautt (1977a), allows direct sampling of hepatic venous blood. In this preparation the inferior vena cava is cannulated and blood returned to the heart from the lower part of the body by connections from the femoral veins to the external jugular veins. The flows and pressures are measured using external probes in the hepatic artery and the portal vein. In both of these types of preparations the liver is not isolated from the general circulation and is innervated. Physiological changes induced elsewhere in the body, for example, increases in heart rate or release of hormones, may affect the liver vasculature. The advantage of these preparations is that they offer a much more physiological environment for experimentation and allow assessment of nerve function.

iii) Transillumination techniques.

These involve direct microscopic observation of the liver vasculature in situ or after excision. Changes in vessel diameter can be directly observed using this technique and it has been successfully employed to observe the effects of nerve stimulation on sinusoid diameter (Koo et al., 1979b, 1979c).

iv) Intravenous injections.

These techniques are the least disruptive when applied to liver circulation. They involves simple intravenous injections of vasoactive substances (for example, cimetidine, Jackson 1981), and measurement of liver blood flow using electromagnetic flow meters. They may therefore be used for studies in humans.

(b) Arterial buffer response.

The hepatic artery and the portal vein do not behave independently of each other. Studies carried out by Burton-Opitz in dogs (1911a), showed that a decrease in portal vein flow may cause an significant increase in hepatic artery flow. This increase in artery flow compensates, in part, for the decrease in portal vein flow, keeping total blood flow constant. Initially this effect was thought to be related to oxygen concentrations within the liver. Experiments

by Lautt et al. (1977b, 1977c, 1980a, 1981), have also demonstrated this effect, known as the "arterial buffer response". Lautt et al. (1977b, 1977c, 1980a, 1981), have shown that the response is independent of the oxygen consumption of the liver. It is believed that the role of this response is to keep total liver blood flow constant so that substances may be removed at a constant rate. The portal vein vasculature has little control over the flow of blood into the portal vein. This is mainly controlled by blood flow to the gut. It is the artery which takes on the role of a buffer to changes in portal venous blood flow. Lautt believes that the mechanism responsible for these changes involves the rate of washout of adenosine. He has shown this to be a potent hepatic artery dilator (Lautt 1984, 1985b). The metabolite washout theory works on the principle that adenosine is being continually produced within the vasculature. The slower the total blood flow the longer the adenosine has to act leading to greater dilation and increasing blood flow. An increased liver blood flow will cause a decrease in the dilator effect of adenosine and so provide a feedback control mechanism for arterial blood flow.

This arterial buffer response may produce physiological changes in the hepatic artery:

portal vein flow ratio. It must also be borne in mind when considering the effects of vasoactive substances in the vascular beds of the hepatic artery and the portal vein.

(c) Vasoactive effects on the vascular bed of the hepatic artery and portal vein.

i)Adrenoreceptors.

Chakravarti et al. (1940), in isolated dog livers, showed that adrenaline causes an increase in hepatic artery and portal vein perfusion pressure. Mesenteric vein injections of adrenaline in rats may also cause an increase in portal vein pressure, Daniel et al. (1951). Using transillumination techniques Seneviratne (1949), and Ho (1972), have demonstrated adrenaline and noradrenaline induced sinusoid constriction. Andrews et al. (1955), noted that in perfused dog livers, injections of both adrenaline and noradrenaline into the hepatic artery or the portal vein increased resistance in either vessel. Using long circuited dog preparations, Green et al. (1959), was able to increase hepatic arterial resistance but could only produce slight changes in portal vein resistance using adrenaline and noradrenaline

injections into the hepatic artery. Introduction of the same substances into the portal vein resulted in an increase in both input vessel resistances. These constrictor responses would seem to be α-adrenoreceptor mediated as they were antagonised by dibenzyline. However, noradrenaline mediated portal vein constriction, during hepatic artery infusion, has been demonstrated by Noguchi et al. (1970), in the isolated perfused rat liver.

Hagino et al. (1974), reported the presence of only α-adrenoreceptors in the rat liver. Hirsch et al. (1976), using dogs, found propranolol to be ineffective at antagonising noradrenaline or adrenaline vasoconstriction in either the portal vein or hepatic artery. Richardson et al. (1977a, 1978), using dogs showed that noradrenaline or adrenaline induced vasoconstriction, in both vessels, was dose related and could be antagonised by the α-adrenoreceptor blocker phentolamine but not by the β-adrenoreceptor blocker propranolol. Studies by Koo et al. (1977), using transillumination techniques in the rat indicate low doses (10^{-9} to 10^{-7} mol/litre) of adrenaline and noradrenaline may induce sinusoid dilation. At higher concentrations a dose related vasoconstriction was obtained which could be antagonised

by the α-adrenoreceptor blocker phenoxybenzamine. Hepatic nerve stimulation may also cause constriction of the vascular bed which may be antagonised by the α-adrenoreceptor blocker phentolamine (Greenway 1979).

β-adrenoreceptor activity has been more difficult to elicit in the liver vasculature. Green et al. (1959), using dogs, could not find any β-receptor activity in either the hepatic artery or portal vein vascular beds. Nylidrin, a β-adrenoreceptor agonist, was shown to produce a β-mediated hepatic artery vasodilation but a vasoconstriction of the portal vein which was potentiated by propranolol, in isolated canine livers (Geumei et al., 1968). Low doses of adrenaline (0.1- 0.2 ug/ml) were found to produce β-adrenoreceptor mediated hepatic artery dilation in cats (Greenway et al., 1969). Using dogs Hirsch et al. (1976), showed that hepatic artery dilation could be produced by hepatic artery or portal vein infusions of the β-adrenoreceptor agonist isoprenaline. Portal vein dilation could not be obtained. The dilation was shown to be β-adrenoreceptor mediated using propranolol. Koo et al. (1977), using transillumination techniques in the rat managed to elicit dilatory responses in sinusoids during 10^{-9} to 10^{-7} mol/litre portal vein infusions of adrenaline and noradrenaline.

The response was found to be β-adrenoreceptor mediated

Studies have been carried out to identify the type of β-adrenoreceptor present in the liver vasculature. Isoprenaline and salbutamol (a β_2 selective agonist) cause vasodilation of the hepatic artery in dogs (Richardson et al., 1977d). This dilation was antagonised by the non-selective β-adrenoreceptor blocking drug propranolol but not by the selective β_1 -adrenoreceptor blocker atenolol. Thus hepatic vasculature β-adrenoreceptors appear to be predominantly of the β_2 subtype. Richardson et al.(1978b), using dogs was unable to elicit any portal vein vasodilation with isoprenaline. Sinusoidal dilation has been shown in the rat during portal vein infusions of isoprenaline and terbutaline (Koo et al., 1979a). Terbutaline is a selective β_2 -adrenoreceptor agonist suggesting β_2 mediated dilation. Propranolol was effective at antagonising the dilation but atenolol was not. Reilly et al. (1981), in rats using microscopic in situ examination showed that isoprenaline can produce both portal vein and sinusoid dilation and using the selective β_1 and β_2-adrenoreceptor blockers (practolol and butoxamine) they found that the majority of receptors may be of the β_2 subtype.

ii) Other receptors.

Seneviratne (1949), Koo et al. (1979a, 1979b, 1979e) have shown the presence of cholinergic receptors in the rat liver sinusoids. Green et al. (1959) found cholinergic receptors in the hepatic artery but not in the portal vein. Histamine receptors have been demonstrated in the sinusoids, (Seneviratne, 1949) and in the hepatic artery and portal vein, (Schwartz, 1970; Richardson et al., 1976, 1977c, 1978a). The receptors are dilatory in the sinusoids and the hepatic artery but produce constriction in the portal vein. Hirsch et al. (1976) and Richardson et al. (1977e, 1978c), have also reported the presence of dopamine receptors in the hepatic artery vasculature of the dog. They remain unconvinced as to the presence of portal vein dopamine receptors. Other vasoactive substances include sodium nitrite (Geumei, 1969b), which was found to produce constriction in both vessels. Bradykinin (Richardson et al., 1977d), has been shown to be a potent hepatic artery vasodilator. 5-Hydroxytryptamine at low doses produces portal vein dilation but at high doses constriction. In the hepatic artery a biphasic dilation followed by a constriction was seen (Richardson et al., 1978a). Glucagon, secretin, pancreozymin and pengastrin all

cause hepatic artery vasodilation (Richardson et al., 1977b).

6) HEPATIC ARTERY AND PORTAL VEIN VASCULATURE INTERACTIONS.

Experiments by Burton-Opitz (1911), in dogs, Condon et al. (1962), in calves and Lautt et al. (1977b, 1977c, 1980a, 1981, 1985a, 1985b), in cats have shown the existence of an interaction between the portal vein flow and the hepatic artery flow known as the hepatic artery buffer response. It has been shown that noradrenaline and adrenaline can increase hepatic artery resistance whether they are infused via the hepatic artery or the portal vein, (Green et al. (1959) and Richarson et al. (1978b). However, injections given directly into the hepatic artery produce a larger constriction than the same dose given via the portal vein. Isoprenaline induced vasodilation of the hepatic artery can also be obtained from both hepatic artery and portal vein infusions (Green et al., 1959 and Hirsch et al., 1976). Portal vein resistance seems to be unaffected by artery infusion. Richardson et al. (1978a), showed that histamine given by the hepatic artery or portal vein produces artery dilation and portal constriction. Angiotensin produces hepatic artery constriction when given by either route (Lautt et al., 1984). Adenosine was shown to be a potent hepatic artery dilator via either route (Lautt et al.,

1984). However, Hirsch et al., (1976) showed dopamine to be equipotent at increasing hepatic arterial resistance through both both routes.

7) DRUG METABOLISM AND CLEARANCE.

Few parent compounds can be directly eliminated from the body and have to undergo biotransformation prior to excretion. This process is known as metabolism and the major site of metabolism is the liver. Hormones such as aldosterone (Camargo et al., 1965), inert dyes such as indocyanine green (Pumgartner et al., 1970) and drugs such as phenacetin (Pang et al., 1978), etidocaine (Tucker et al., 1977), imipramine, (Gram et al., 1975), all undergo liver metabolism prior to removal.

The purpose of drug metabolism is to render parent compounds more water soluble and more amenable to excretion. For most drugs the process can involve two stages , Phase I and Phase II metabolism.

(a) Drug Metabolism.

Phase I metabolism occurs when the basic chemical structure of the parent drug is altered. This is usually achieved by the introduction of polar groups by oxidation, reduction, hydrolysis or by the removal of alkyl groups to "uncover" potential polar groups. The process involves the haemoprotein cytochrome P_{450} of which there are several subtypes. The cytochrome is reduced by NADPH-cytochrome c reduc-

tase to produce active oxygen complexes (Brodie et al., 1958 and Cooper, 1965). The process is localised within the microsomal fraction of cells (Gillette, 1971), and although skin, lung and other tissues may exhibit cytochrome P_{450} activity, (Baron et al., in press), the liver is the main site of metabolism.

Phase II metabolism occurs when the parent drug or its metabolites are conjugated with one of several endogenous substrates such as glucuronic acid, sulphate, glycine, glutamine or acetate. Glucuronidation and sulphation are two types of conjugation commonly seen in drug metabolism. Glucuronidation is localised in the liver microsomal fraction (Isselbacher et al., 1962). The process involves the formation of UDP-glucuronic acid from glucose-1-phosphate and the transfer of the glucuronic acid molecule to the drug; a reaction catalysed by the enzyme glucuronyl transferase. Sulphation mainly occurs in the cell cystol, (Nose et al., 1958). Sulphate is first converted to the active 3-phosphoadenosine-5-phosphosulphate form (PAPS). The sulphate is then transferred to the drug molecule by sulphotransferase. Not all drugs undergo Phase I metabolism prior to conjugation, for example, digitoxin is almost entirely excreted as conjugates of the parent drug.

(b) Clearance of drugs.

The clearance of a drug represents the efficiency with which it is removed by the eliminating organs. Total body clearance can be defined as the fraction of the apparent volume of distribution cleared of drug by the body per unit time. Apparent volume of distribution may expressed in terms of blood volume or the plasma volume. Total body clearance is the sum of clearances by each eliminating organ, for example, liver, lung or kidney. These clearances fall into two categories; renal clearance and metabolic clearance. Renal clearance is defined as the urinary drug excretion divided by plasma concentration. Since the main site of metabolism for most drugs is the liver, the metabolic clearance is normally equivalent to hepatic clearance.

Hepatic clearance, (Cl_h) is a function of liver blood flow and the ability of the liver to extract the drug as it passes through the hepatic sinusoids.

$$Cl_H = Q \times E \qquad \text{Equation 1.7.1}$$

Q = liver blood flow

E = extraction ratio of the drug

E may be defined as the ability of the liver to irreversibly remove a given quantity of drug in one passage through the liver.

$$E = \frac{(C_{IN} - C_{OUT})}{C_{IN}} \qquad \text{Equation 1.7.2}$$

C_{IN} = Hepatic artery and portal vein input concentration of drug

C_{OUT} = Hepatic venous output concentration

A quantitative approach to the removal process is obtained by considering the total intrinsic clearance, (Cl_{INT}) of the liver. This is the maximal ability of the liver to remove drug irreversibly when flow is not limiting. Using this parameter:

$$Cl_H = \frac{(Q_H \times Cl_{INT})}{(Q_H + Cl_{INT})} \qquad \text{Equation 1.7.3}$$

Many drugs are bound to plasma proteins and pass through the liver in both the bound and free forms. Normally only free drugs may be metabolised but where the avidity of the hepatic extraction process is high, drug may be stripped from the binding proteins during passage through the liver. Uptake of drug may

be restricted to free drug where protein binding is strong but may be unrestricted where bound drug may be displaced by the extraction process. The fraction of unbound drug in the blood is usually represented by fb. Thus:-

$$Cl_H = Q_H \times \left[\frac{(fb \times Cl`_{INT})}{(Q_H + fb \times Cl`_{INT})} \right] \qquad \text{Equation 1.7.4}$$

Where $Cl`_{INT}$ is the intrinsic clearance of unbound drug. If $Cl`_{INT}$ greatly exceeds Q_H and binding is constant the part of the equation in square brackets approximates to unity and equation 1.7.4 can simplified to:-

$$Cl_H = Q_H \qquad \text{Equation 1.7.5}$$

The hepatic clearance of drugs which show a high intrinsic clearance will be affected by changes in liver blood flow. Whereas for drugs showing a lower intrinsic clearance the approximation does not hold and these drugs will be affected by changes in the maximum veocity of the extraction process. Changes in the V_{max} of the metabolism process may be achieved using cytochrome P_{450} inducers.

Drugs may be considered to be relatively flow dependent or flow independent. Drugs which show a high Cl_{INT} by the liver and are thus flow dependent are propranolol, (Wood et al., 1979; Branch et al., 1973, oxprenolol, (Mason et al., 1976), metoprolol, (Borg et al., 1975) and lignocaine, (Branch et al.,1973 and Ahmad et al., 1983). Drugs showing a low extraction ratio include diphenyl-hydantoin (Shand et al., 1975) and warfarin (Esquivel et al., 1978).

Drugs which have high intrinsic clearances such as those outlined above may be used as tools to measure liver blood flow since their clearances are flow dependent. This is only true if the drugs have a constant extraction ratio and are thus flow dependent.

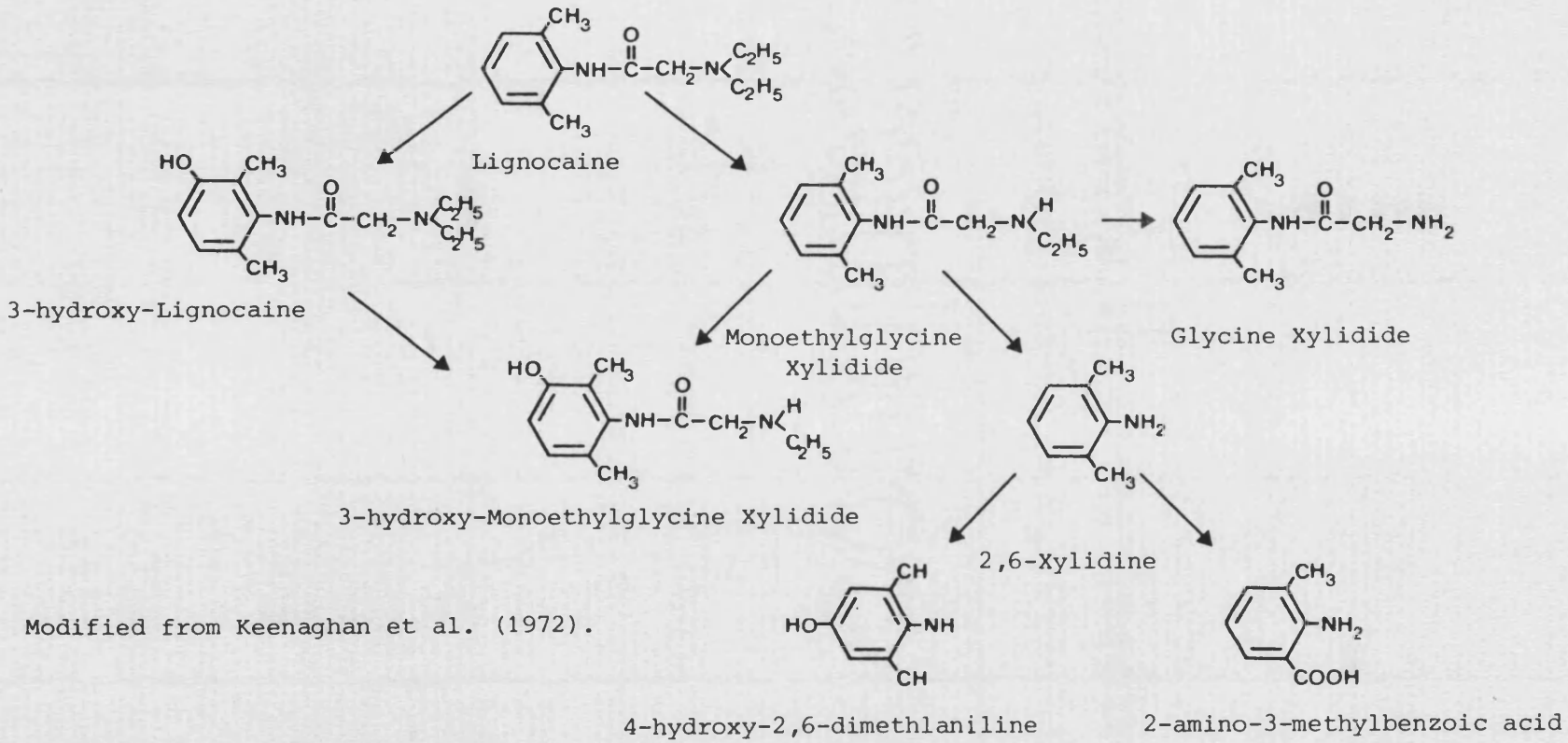
8) SCOPE OF THE STUDY.

The liver has been shown to be a complex structure. The functional unit, the acinus, shows a degree of heterogeneity, both in structure and function. The vascular connections to the acinus have also been shown to be zone dependent. In addition the two liver blood supplies, the hepatic artery and the portal vein may differ in their routes through the liver and their enzyme environments. The possibility exists that drugs may be metabolised differently by the two routes. The hepatic artery and the portal vein also respond differently to vasoactive substances and as such the physiological normal hepatic artery portal vein flow ratio may be altered by these substances.

The present study investigates the effect of changes in the hepatic artery portal vein (HA:PV), flow ratio and pressure on the metabolism of the drug lignocaine in the perfused rat liver. Lignocaine was used as a model drug as it is highly extracted in both rats and humans, (Branch et al., 1973; Shand et al. 1975; Ahmad et al., 1983; Lennard et al., 1983). It is therefore a liver blood flow dependent drug (Nies et al., 1976). Its metabolism in the rat (Hollinger et al., 1960; Keenaghan et al., 1972; Nyberg et al., 1977) and in man (Beckett et al.,

1966; Hermansson et al., 1980; Drayer et al., 1983), has been well documented (Fig. 1.8.1). Also work by Ahmad et al.(1983) has shown that the extraction ratio is dependent on the hepatic artery to portal vein flow ratio.

Figure 1.8.1. The Known Metabolism of Lignocaine



Modified from Keenaghan et al. (1972).

CHAPTER 2

MATERIALS AND METHODS

1) GENERAL CHEMICALS

Calcium Chloride	- S.L.R. Grade Fisons Loughborough
Glucose	- S.L.R. Grade Fisons Loughborough
Hydrochloric Acid	- S.L.R. Grade Fisons Loughborough
Magnesium Sulphate Heptachloride	- S.L.R. Grade Fisons Loughborough
Potassium Chloride	- S.L.R. Grade Fisons Loughborough
Potassium diHydrogen-Orthophosphate	- S.L.R. Grade Fisons Loughborough
Potassium Hydroxide	- S.L.R. Grade Fisons Loughborough
Sodium bicarbonate	- S.L.R. Grade Fisons Loughborough
Sodium Chloride	- S.L.R. Grade Fisons Loughborough

All chemicals were made up or diluted with double distilled water.

Solvents

Acetonitrile	- H.P.L.C. Grade Fisons Loughborough
Dichloromethane	- H.P.L.C. Grade Fisons Loughborough
Diethyl Ether	- S.L.R. Grade Fisons Loughborough
Methanol	- H.P.L.C. Grade Fisons Loughborough

2) DRUGS

Adrenaline - Epinephrine bitartrate, Sigma, England

Atenolol - Atenolol hydrochloride, I.C.I. England

Bupivicaine - Bupivicaine hydrochloride, (Marcaine Plain), Duncan Flockhart, Greenford, Middlesex.

Clenbuterol - Clenbuterol hydrochloride, Dr. Karl Thomae, G.M.B.H. Germany.

Dobutamine - Dobutamine hydrochloride (Dobutrex), Lilly, England.

Desbutyl-Bupivicaine - AB Borfors, Borfors, Sweden

Hydralazine - Hydralazine hydrochloride, Sigma, England.

I.C.I.118 551 - I.C.I. England.

Isoprenaline - Isoprenaline hydrochloride (Saventrine), Pharmax Ltd., Kent, England.

Indocyanine Green - Sigma, England.

Lignocaine - Lignocaine hydrochloride, (Xylotox) Pharmceutical Manufactures, Epsom, Surrey, England.

^{14}C Lignocaine - Lignocaine hydrochloride, New England Nuclear, Dupont, Southampton, England.

Metoprolol - Metoprolol Tartrate, Sigma, England.

norAdrenaline - Arternolol ±bitartrate, Sigma, England

Oxprenolol - Oxprenolol hyrochloride, Sigma, England.

Phentolamine - Phentolamine Mesylate, (Rogitine), Ciba, Sussex, England.

Propranolol - Propranolol hydrochloride, I.C.I. England.

3) ANIMALS

In all experiments male Wistar albino rats were used. These were C.F.H.B. Bath University strain. Rats for perfusion were normally allowed to grow up to 450g. Rats for isolated heart experiments weighed between 200g and 250g. All animals were kept in cages of no more than six and were fed on normal laboratory chow. Prior to all experiments the animals were allowed free access to food and water.

4) APPARATUS USED FOR LIVER PERFUSION

All liver perfusion experiments were carried out in a perfusion cabinet. This consisted of a metal frame eighteen inches wide, thirty inches long and thirty six inches high. Attached to the back and sides of the cabinet were sheets of plywood 1cm thick. These were covered on both sides with Benchkote, (Whatman, Maidstone,England), to facilitate cleaning. The top of the cabinet consisted of three perspex sheets placed side by side, which were easily removable and allowed manipulation within the cabinet. The front of the perfusion cabinet was divided into three horizontal sections. Each of these sections could be accessed during the experiment using one of three perspex doors attached to the metal frame of the cabinet. These were hinged on their lower surface and when opened were supported by chains on each side. In the closed position they were held in place by clips on the side of the cabinet. This facility allowed manipulation within the cabinet with minimum heat loss and disturbance.

The cabinet was thermostatically controlled at $37^{o}C \pm 2^{o}C$. This was achieved by a combination of four electric lamps. Two 100W lamps were attached to the back of the lower section of the

cabinet. These were continuously on during perfusion experiments but could be adjusted by a potentiometer placed on the outside of the cabinet. A further two 100W lamps were placed in the right upper section where the perfused rat was placed. These provided a finer control of temperature within this area of the cabinet and also a good light source for surgery. These two lights were automatically controlled by a platinum resistance temperature controller, (R.S. components, Northants, England), linked to a platinum thermocouple (R.S. components, Northants, England). This system was calibrated using a 50 uA - O - 50 uA voltmeter, (R.S. components, Northants, England). The temperature controller and the voltmeter were housed in a box attached to the outside of the cabinet. This system was sufficiently accurate to provide overall cabinet temperature control of $\pm 2^{o}C$ and finer control of $\pm 1.5^{o}C$ around the rat. The floor on the right hand side of the top section of the perfusion cabinet consisted of a perspex sheet which was used to support the operating board. To the left of the cabinet`s top and middle sections an internal metal frame was built which supported three water jacket heaters, two mercury manometers and two pressure transducers. Also supported by this frame and

extending in to the right middle section of the cabinet were two oxygenators. The floor of the left middle section consisted of a sheet of perspex and supported two magnetic stirrers. The floor in the lower section of the cabinet consisted of two sections of plywood covered with Benchkote, (Whatman, Maidstone, England). Placed on this floor was an electric fan and a water heater. The fan was kept permanently running throughout the perfusion experiments. The heater was a Gallenkamp Thermostirrer 85 (Gallenkamp, Loughborough, England) and was used to heat water for the jacket heaters. The Thermostirrer was placed in a 3 litre plastic beaker and was set at 37^{o}C.

Supplies of perfusion medium to the hepatic artery and the portal vein were entirely separate each having its own pump, oxygenator, heater and bubble trap. The perfusion medium was kept in glass beakers. Usually only one reservoir was needed but where experiments required separate reservoirs two beakers were used each having its own magnetic stirrer. Medium from the reservoirs was sucked up by peristaltic pumps, (Wastson-Marlow, Falmouth, England) these were situated outside the cabinet and were preset to deliver the correct flow to each supply. Total flow rate to the liver was always kept

at a constant 10 ml/min, however, the ratio of hepatic artery to portal venous flow was varied. Where one supply exceeded 5 ml/min the medium in that supply passed through a jacket heater immediately after leaving the reservoir. This ensured sufficient warming of the medium at the higher flow rates.

The medium for both the hepatic artery and portal vein supplies then passed into oxygenators. These consisted of two 500 ml round bottomed flasks each of which contained 25 feet of medical grade silastic tubing, (Dow Corning, U.K.). The flasks contained a mixture of 95% Oxygen and 5% Carbon Dioxide at a pressure of 50mmHg. The gas was supplied at a rate of 100 ml/sec (measured at atmospheric pressure), by a cylinder outside the cabinet. The oxygenator was sealed by a rubber bung placed in the top of the flask; a vent in the bung controlled the gas outflow. A further three holes in the bung were made, one for the gas input and the others to allow the tubing carrying the perfusion medium to pass in and out of the oxygenator. Silastic tubing is porous to oxygen therefore fluid flowing within the tubing in an oxygen rich environment becomes highly oxygenated. The system has advantages over gas bubbling oxygenators when the perfusion medium contains red blood cells,

as it greatly reduces haemolysis due to turbulance.

After passing through the oxygenators the medium in each supply passed out of the cabinet, through an external pump and returned to pass through a water jacket heater situated inside the cabinet. The perfusion medium passed into a bubble trap. This consisted of a 2 ml syringe from which the plunger had been removed and the rubber end of the plunger sealed into the top of the syringe. Inserted through this rubber seal were two 15 gauge Luer stub adapters. The seals were made airtight and a further connector was attached to the nozzle of each bubble trap. The traps were positioned at 45^{o} to the vertical with the nozzle pointing downwards; in this position they were found to remove all air bubbles from the medium. One of the top Luer adapters formed the input and the nozzle end adapter the output. The third adapter was connected to the pressure control and measurement apparatus.

The pressure within each trap could be controlled using a bulb with an air flow valve. This system was used to ensure that the level of fluid in each bubble trap was sufficient to prevent air passing in to the outlet. Connected to this pressure control system was a mercury manometer which was used to give

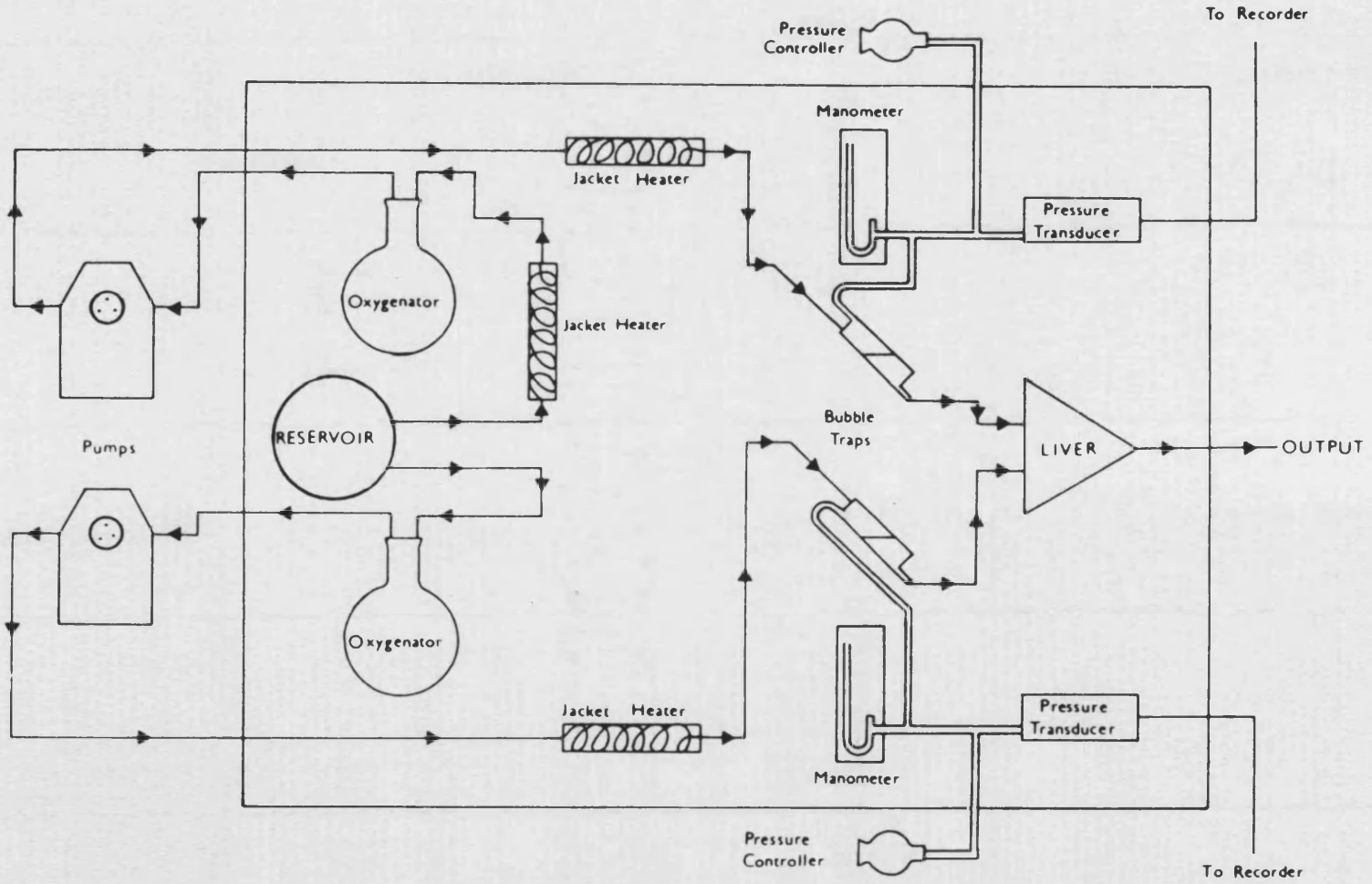
quick visual readings of the pressure within the supply. Pressure in each supply was also monitored using a Bell Howell type 4-422-0001 pressure transducer, (Bell Howell, Basingstoke, England). The two transducers (one for each supply), were in turn connected to a Devices M2P Amplifier fitted with two DC.2D and two .1C modules (Devices, Hertfordshire, England). This amplifier was connected to a Devices M2R duel channel recorder fitted with a DC.5 and a DC5H module, (Devices, Hertfordshire, England). This system allowed the measurement of the small pressure changes seen in the portal vein and the larger pressure changes seen in the hepatic artery. The dual recorder provided a permanent record of the perfusion pressures in each supply throughout the experiment.

In experiments where no vasoactive agents were to be infused, output from the bubble traps was connected directly to the artery or the portal vein cannulae. However, for experiments where vasoactive agents were to be used, an infusion port was placed between the bubble trap and the hepatic artery and portal vein cannulae. This port consisted of a modified plastic three way connector with a self sealing rubber membrane secured over one of the inputs. The two other branches provided the input and output

connections for the perfusion medium. Vasoactive substances could be introduced into the supply via this port either by injection or by slow infusion. The slow infusion apparatus employed consisted of a slow infusion pump, (Scientific Research Instruments No. 5200, Kent, England.), to which 5 ml syringes were attached. These were connected to a 24 gauge needle unit from a 24 gauge catheter placement unit, (Quickcath, Dupont Travenol Laboratories, Ireland). The needle unit was forced into a length of non-sterile portex nylon tubing X diameter (800/110/140/100, Portex, Kent, England). The other end of this tubing was connected to a fine hollow needle which was used to puncture the rubber membrane placed over one branch of the three way connector. This, allowed agents to mix with the perfusion medium of each supply.

Figure 2.4.1. A Photograph to Show the Apparatus Used for Isolated Liver Perfusion in Situ.

Figure 2.4.2. A Diagram to Show the Apparatus Used for Isolated Liver Perfusion in Situ.



5) PREPARATION OF THE PERFUSION MEDIUM

The perfusion medium consisted of red blood cells suspended in Krebs buffer, (Appendix 1). The pH of the medium was adjusted, where necessary, to pH 7.4 using 0.1 M hydrochloric acid or 0.1 M potassium hydroxide. The cells were out-dated human red blood cells and obtained from the Area Blood Transfusion Centre in Bristol. These cells deteriorated rapidly even when refrigerated and were always used within one week of delivery.

The cells were washed with Krebs buffer prior to being perfused. This process involved mixing 10 ml of cells with 20 ml of Krebs buffer in 30 ml glass centrifuge tubes, inverting the tubes gently then centrifuging for ten minutes at 2000g in an IEC Centra-7 centrifuge, (Damon, Bedford UK). The supernatant was then removed and the process repeated until the supernatant resembled the clarity of the Krebs buffer. The first three washes used Krebs buffer containing heparin, 100 units per ml, added to prevent clotting. The cells were normally washed at least six times and all the subsequent washing was carried out with heparin free Krebs buffer.

The packed washed red blood cells were then added to the perfusion medium of Krebs

buffer to give a final haemoglobin concentration of 5 to 6 g/100ml, as measured by a commercial test kit, (Sigma, Dorset, England). The perfusion medium was found to be sensitive to storage, even if refrigerated ($4^{o}C$) and was freshly prepared for each perfusion.

In experiments in which lignocaine and its metabolites were investigated under changing flow conditions the substances were added directly to this medium. However, for experiments to study the effects of vasoactive substances, the agents were infused in to the perfusion medium through an infusion port as already described.

6) OPERATIVE PROCEDURES.

For the perfusion experiments male albino wistar rats, Bath University (CFHB) strain, weighing between 400-450g were used. The animals were allowed free access to water and food prior to the experiment. They were anaesthetised with 6% pentabarbitone (Sagatal, May and Baker, Dagenham, England) 70mg/kg given intraperitoneally. This resulted in full surgical anaesthesia with loss of corneal reflex. After this had been achieved the rat was transferred to a 14 inches by 14 inches perspex operating board. This allowed the initial procedures to be carried out on the bench and transferal of the animal to the cabinet for the final cannulations. The time that the liver was deprived of a blood supply could be kept to a minimum if the final operative procedures were performed within the the perfusion cabinet.

After positioning the animal dorsal side down on the operating board, a midline incision was made in the skin from the lower abdomen to the neck region. The abdominal contents were exposed by a further incision made along the linea alba to the xiphoid process. The major abdominal vessels were clamped off near the xiphoid process to reduce bleeding. Two mid-transverse incisions in the body wall to the right and

left of the midline were made just below the diaphragm and below the clamps. A further two mid-transverse incisions in the body wall to the right and left were made at a level below the kidneys. The two tissue flaps were then folded back.

The abdominal contents were gently displaced to the animals left hand side to expose the major vessels of the abdomen. At this point, in initial experiments, a P10 cannula (Portex, Kent, England) was placed in the bile duct. However, it was found that the production of bile during perfusion experiments was extremely small and insufficient for analysis. In subsequent preparations the bile duct was ligatured.

The thin strands of the hepato-renal ligament were cut exposing the vena cava above the right renal vein. A loose ligature was then placed around the vena cava at this point. The connective tissue surrounding the portal vein was cleared and two loose ligatures placed around the vessel, one a few millimeters below its entry into the liver and the other above the convergence of the splenic and gastric veins.

The aorta was then located and the path of the coeliac artery cleared. The gastric arteries,

gastroduodenal artery and lineal artery were all isolated and ligatured. At this point all blood passing along the coeliac artery now flowed into the liver by the hepatic artery. Two loose ligatures were placed around the coeliac artery, one as close as possible to its divergence from the aorta, the other a few millimeters below its divergence into the left and right hepatic arteries. Using a curved, fine Spencer Wells artery clamp, the artery was clamped just above the lower ligature. The artery could then be straightened by manoeuvring the clamp, facilitating cannulation of the vessel. A cannula was then placed in the artery above the clamp using a 24 gauge catheter placement unit (Quick Cath, Dupont Travenol Labs, Ireland). This was tied in place using the upper loose ligature. Blood was allowed to flow back along the cannula and then 1 ml of Krebs buffer containing 1000 units of heparin was injected into the cannula to prevent any clots forming and to ensure the viability of the cannula. The second loose ligature was then tied off, the clamp removed and a bung placed in the cannula to prevent blood loss.

At this point the rat was transferred to the cabinet. Both perfusion pumps were switched on and the medium from the outflow allowed to drip into a

beaker. This ensured that no air bubbles would be present in the tubing when the two supplies were connected. A cannula was then placed in the portal vein between the two loose ligatures, using a 20 gauge catheter placement unit, (Quick Cath, Dupont Travenol Labs, Ireland). This was tied in place using the upper loose ligature. The lower ligature was tied to prevent bleeding from the portal vein.

The liver was now devoid of any blood supply and it was essential to perform the next step within one or two minutes. This involved opening the chest cavity with two incisions through the rib cage and clamping the flap back after freeing it from the diaphragm. The vena cava was then cleared from connective tissue and separated from the vagus nerve. Two loose ligatures were placed around the vessel and the one nearer the heart tied off. A 14 gauge catheter placement unit, (Venflow Viggo, Helsingborg, Sweden) was then used to cannulate the inferior vena cava. This was tied in position using the other loose ligature.

The two supplies were immediately connected to the portal vein and hepatic artery cannulae and an output connected to the vena cava. The loose ligature which had been placed around the posterior

vena cava above the right renal vein was tied off so that all liver output was directed out of the inferior vena cava cannula. If the proceedure had been sucessful after approximately one minute of perfusion the liver should become an even pink colour. If any dark blue blotches of anoxic tissue were seen the preparation was discarded. Also, any livers which showed a high initial pressure after five minutes, that is greater than 130 mmHg in the artery or 30 mmHg in the portal vein, were discarded. If these two criteria were normal then the flaps of tissue were replaced to their normal position as far as possible and the liver covered with a piece of cotton gauze soaked in the perfusion medium to prevent drying out. The preparation was then left running for 20 minutes to equilibrate and permit flow and pressure readings to be taken.

7) ANALYSIS OF LIGNOCAINE AND ITS METABOLITES FROM PERFUSION EXPERIMENTS.

(a) Collection and treatment of samples.

Samples were collected from the vena cava output cannula. A piece of tubing passed outside the cabinet to allow collection of samples with the minimum of disturbance to the internal environment of the cabinet. Perfusate was collected directly into a measuring cylinder to allow measurement of flow rate. Samples from this were then decanted into 15 ml centifuge tubes and spun at 2000g (IEC Centra-7, Damon, Bedford, UK). The supernatant was transferred into glass vials and frozen at -20^{o}C or analysed that day. It was found that the samples were stable for two weeks when frozen, but analysis was usually performed within four days of storage. In order to calculate extraction ratios, liver input samples were also taken. These were obtained after completion of the perfusion experiments. The two supplies were allowed to drip into a beaker and the sample obtained was then treated in the manner as effluent perfusate samples. These liver input samples were also used as controls in the analysis of metabolite concentrations.

(b)High Performance Liquid Chromatography (HPLC) Apparatus.

Lignocaine and its metabolites were all measured using HPLC. The same apparatus being used for each assay although different mobile phases were used. The apparatus consisted of a 7125 type Rheodyne injection port fitted with a 100 ul injection loop (Rheodyne, California, USA), a Constametric III pump (LDC Instruments, Florida, USA), a block heater (Jones Chromatography, Glamorgan, Wales), a Spectromonitor III ultraviolet detection system (LDC Instruments, Florida, USA) and a CR650S recorder (JJ Instruments, Southampton). The separating column consisted a 25 cm, 5 um hypersil ODS reversed phase analytical column fitted to a 5 cm, 5 um hypersil ODS reversed phase precolumn (both from Jones Chromatography, Glamorgan, Wales). The main column was kept at 25^{o}C using the block heater, the precolumn was not temperature controlled. All high pressure tubing and joints were stainless steel, all other connections were made with PTFE tubing resistant to solvent attack.

(c) Analysis of Lignocaine.

5 ml aliquots of perfusate supernatant were used to measure both liver input and output lignocaine concentrations. Treatment with sulphatase (S 3009, Sigma, Dorset, England) and Glucurase (G 4882 Sigma, Dorset, England) at pH 5 for 16 hours, had no effects on both input or output lignocaine concentrations. This revealed that no sulphate or glucuronide of lignocaine were formed. In subsequent lignocaine measurements no deconjugation step was necessary.
To each 5 ml aliquot lignocaine sample, 60 ul of 250 ug/ml bupivicaine hydrochloride solution was added as an internal standard. These solutions were then adjusted to pH 11 using 1M hydrochloric acid and 1M potassium hydroxide. 5 ml of diethyl ether was then added to each sample. The solutions were mixed on a rotary mixer, (Luckman Ltd., Sussex, England) for 20 minutes at 1 cycle per second. The tubes were then centrifuged for 5 minutes at 2000g (IEC Centra-7, Damon, Bedford, UK) to separate the mixture into two layers. The diethyl ether layer was removed from each sample using a Pasteur pippette and placed in a clean tube. These tubes were then placed in a water bath set at 40^{o}C and the solvent evaporated using compressed air jets. The extraction process was repeated on each sample to

ensure good recovery. Approximately 90% of the lignocaine could be extracted from the sample by this method.

The extracted dry samples were then mixed with 150 ul mobile phase after the sample had dissolved 100 ul of the solution was injected on to the column using a Hamilton syringe (Anachem, Luton, England). For lignocaine analysis the mobile phase consisted of 40% methanol, 30% acetonitrile, 30% 0.05M potassium dihydrogen orthophosphate buffer. The mobile phase was adjusted to pH 6.90 using 1M hydrochloric acid or 1M potassium hydroxide. The mobile phase was degassed by bubbling helium through the mixture for 10 minutes. The mobile phase was passed through the column at a flow rate of 1.50 ml/min.

A slight adjustment was made to this method for samples which contained phentolamine as well as lignocaine. The phentolamine was found to interfere with the lignocaine peak and a mobile phase of 30% acetonitrile, 30% methanol and 40% 0.05M potassium dihydrogen orthophosphate buffer pH 6.9 was used under the sample conditions as described above.

(d) Analysis of metabolites.

The concentrations of metabolites were determined using the same HPLC apparatus as that used in the lignocaine assay. Four metabolites and an intnal standard were measured in the same assay. The metabolites were 3-hydroxylignocaine, (3-OH LIG), 3-hydroxymonoethylglycine xylidide, (3-OH MEGX), monoethylglycine xylidide, (MEGX) and glycine xylidide, (GX); the internal standard being desbutyl-bupivicaine. Conjugated metabolite concentrations were determined after selective enzyme deconjugation. The various treatments used yielded the free forms of the metabolites which could be measured using the same HPLC assay.

5.2 ml aliquots of perfusate supernatant were taken, three from each sample. One aliquot underwent treatment to deconjugate both sulphate and glucuronide conjugates prior to analysis by HPLC. The process involved adjusting the solution to pH 5 using 1M hydrochloric acid or 1M potassium hydroxide and adding 50 ul of Glucurase, (G 4882, Sigma, Dorset, England) and 50 ul Sulphatase solution (S 3009, Sigma, Dorset, England). The solution was allowed to incubate at 37^{o}C in a shaking water bath for 16 hours.

To determine metabolite sulphate concentrations, a second 5.2 ml aliquot was adjusted to pH 5 but only sulphatase was added as described above. To inhibit any glucurase activity in the solution, 200 ul of 0.2M D-saccharic acid 1,4 lactone (Sigma, Dorset, England) was added. This concentration was found to inhibit almost all glucurase activity but would allow sulphatases to work. The second solution was also incubated at 37^{o}C in a shaking water bath for 16 hours.

A third aliquot of 5.2 ml was taken and adjusted to pH 5 and incubated at 37^{o}C for 16 hours without any deconjugating enzymes present to determine the levels of unconjugated or free metabolites in the perfusate. The system of selective deconjugation results in free metabolites which can be measured by the same HPLC method. Using this assay sulphate metabolites are determined from the concentrations seen after sulphate deconjugation minus the unconjugated metabolites. Glucuronide conjugates are determined from the metabolite levels seen after both glucuronide and sulphate deconjugation minus the levels after sulphate deconjugation.

After selective enzyme treatment all samples were treated by the same extraction method.

The samples were first spun down at 2000g (IEC Centra 7, Damon, Bedford, UK) for 10 minutes. 5 ml of the supernatant was then removed and placed in a separate tube to which 50 ul of a 250 ug/ml solution of desbutyl-bupivicaine was added as an internal standard. The solutions were adjusted to pH 11 using 1M potassium hydroxide. 5 ml of dichloromethane was then added to each tube. The tubes were stoppered with teflon coated screw caps and mixed in a rotary mixer (Luckman Ltd, Sussex) at 1 cycle per second for 20 minutes. The samples were then centrifuged at 2000g (IEC Centra-7, Damon, Bedford, UK) for 5 minutes. Often in this process the dichloromethane layer became white and gelatinous. It was found that by repeated shaking and centrifugation a clear liquid layer of dichloromethane could be obtained. As the dichloromethane is denser than water it forms a layer in the bottom of the tube. It was removed using long Pasteur pippettes and was passed through a type 1 paper filter (Whatman, Maidstone, England) which had been soaked in dichloromethane. This removed any particulate matter sucked up with the solvent. The dichloromethane was filtered into fresh tubes which were transferred to a water bath at 45^{o}C. The solvent was evaporated using jets of compressed air. The extraction process was repeated

for each sample to ensure good extraction of the chemicals. After extraction the dry samples were mixed with 150 ul of mobile phase and 100 ul injected onto the column using a Hamilton syringe, (Anachem, Luton, England). It was found that all four metabolites and the internal standard could be separated using a mobile phase of 14% acetonitrile and 86% 0.05M potassium dihydrogen orthophosphate buffer. The mobile phase was adjusted to pH 6.0 using 1M hydrochloric acid or 1M potassium hydroxide. The mobile phase was degassed by bubbling helium through the liquid for 10 minutes. The initial flow rate used in the assay was 1.5 ml/min, with an increase to 2 ml/min 7 minutes after sample injection.

(e) Calculation of lignocaine and metabolite concentrations in perfusate.

Calculation of concentrations of the various chemicals were made from the ratio of the peak height of the compound against the peak height of the internal standard. These ratios were then compared with standard curves of peak height ratios against concentration. In order to produce these standard curves solutions of lignocaine, each metabolite and internal standards of known concentration were made using

standard compounds. Lignocaine was obtained from The Pharmaceutical Manufacturing Company, (Epsom, Surrey, England). The metabolites, 3-hydroxylignocaine (3-OH LIG), 3-hydroxymonoethylglycine xylidide, (3-OH MEGX), monoethylglycine xylidide, (MEGX) and glycine xylidide, (all as hydrochlorides), were gifts from Astra, UK. Bupivicaine hydrochloride, (Marcain Plain) was obtained from Duncan Flockhart, (Middlesex, UK) and desbutyl-bupivicaine from A. B. Borfors, (Borfors, Sweden).

To obtain standard peak height ratio vs. concentration curves, known concentrations of these solutions were independently made up in Krebs buffer to a final volume of 5.2 ml. To these solutions 1 ml of washed packed red blood cells was added as prepared by the method previously described. These samples were incubated at 37^{o}C for 1 hour in a shaking water bath. On removal they were spun at 2000g (IEC Centra-7, Damon, Bedford, UK) and 5 ml samples of supernatant removed. To lignocaine metabolite solutions 50 ul of 250 mg/ml solution of desbutyl-bupivicaine was added as an internal standard. To solutions of ligocaine 60 ul of bupivicaine was added as an internal standard the relevant extraction procedure was then performed on the sample to prepare it for HPLC anal-

ysis. Both the extraction procedure and the analysis were exactly the same as described previously for perfusate samples. From the ratios of peak height of known standard against internal standard, graphs were drawn of peak height ratio against free base concentration in ng/ml, (Figs. 2.7.5; 2.7.6; 2.7.7; 2.7.8). In all cases these showed a high degree of linear correlation (>0.99).

Two standard curves were constructed for lignocaine solutions. A high range (Fig. 2.5.3) so that lignocaine input concentrations could be determined and a low range (Fig. 2.5.4) so that low liver output levels could be calculated. In experiments where input concentrations of metabolites were infused in similar molar concentrations to those used with lignocaine, the concentration range of the existing standard curves was extended to enable input concentrations to be calculated. These higher metabolite concentrations closely followed the linearity of the existing peak height ratio against concentration graphs.

Figure 2.7.1

A Typical Lignocaine High Performance Liquid Chromatogram

KEY

"a" = Lignocaine

Attenuation X 0.2

"b" = Bupivicaine

Attenuation X 0.5

Lignocaine concentration

342 µg/l

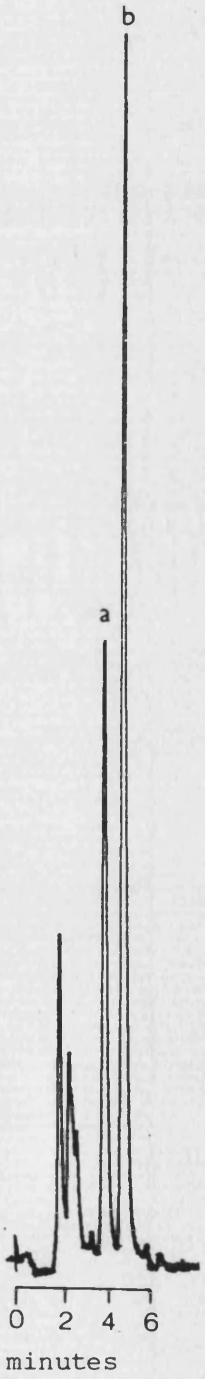


Figure 2.7.2

A Typical Lignocaine Metabolite High Performance Liquid Chromatogram

KEY

"a" = 3-OH MEGX 1550 ng/ml.
Attenuation X 0.2

"b" = 3-OH Lig 420 ng/ml
Attenuation X 0.1

"c" = GX 450 ng/ml
Attenuation X 0.05

"d" = MEGX 240 ng/ml
Attenuation X 0.05

"e" = Desbutyl-Bupivicaine.
Attenuation X 0.1

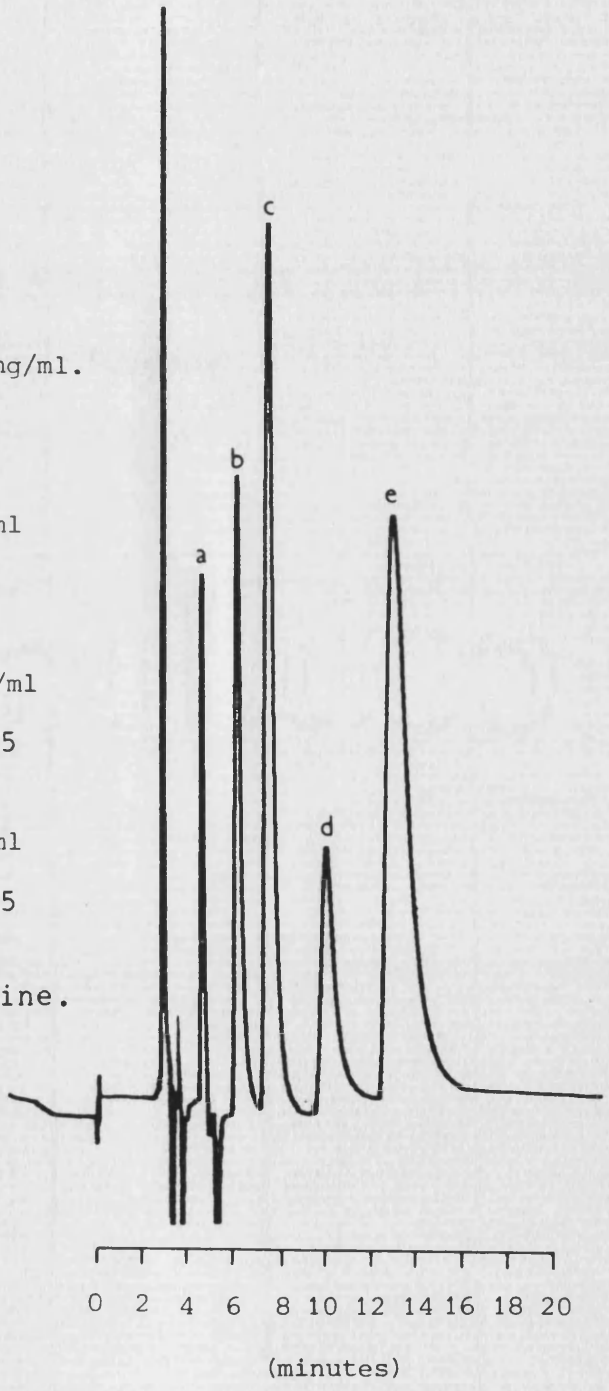


Figure 2.7.3

Lignocaine Standard Curve (High Range).

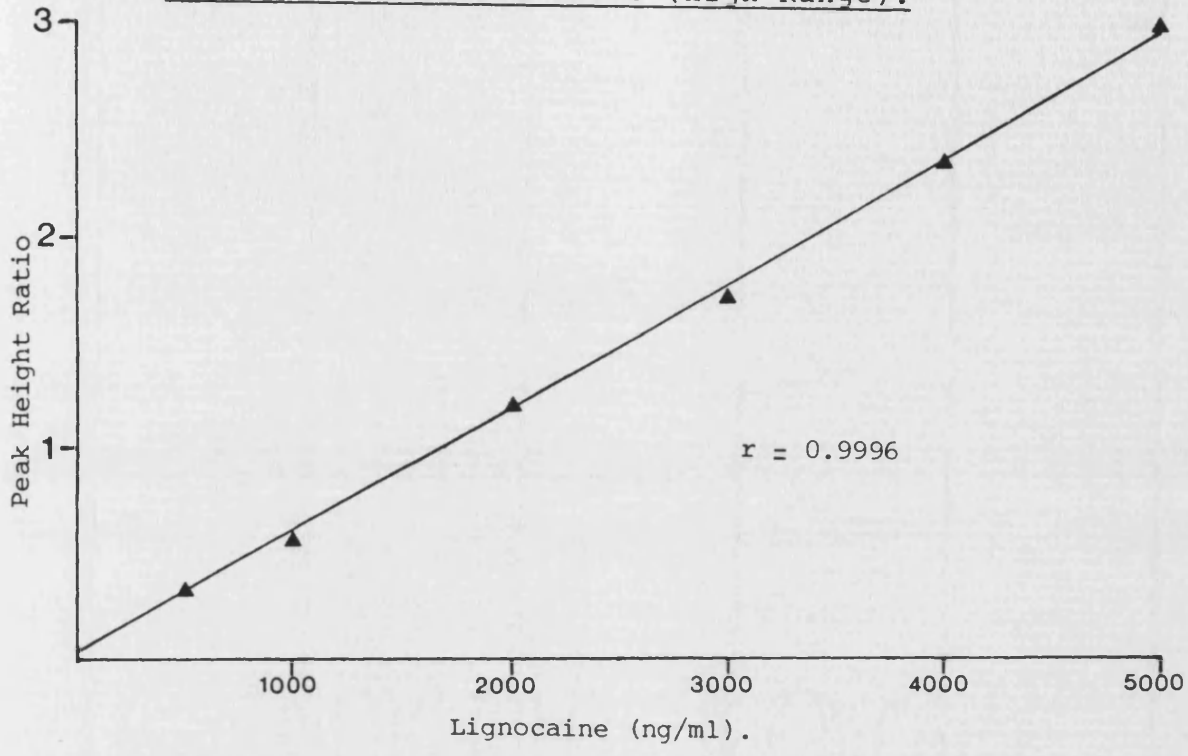


Figure 2.7.4

Lignocaine Standard Curve (Low Range).

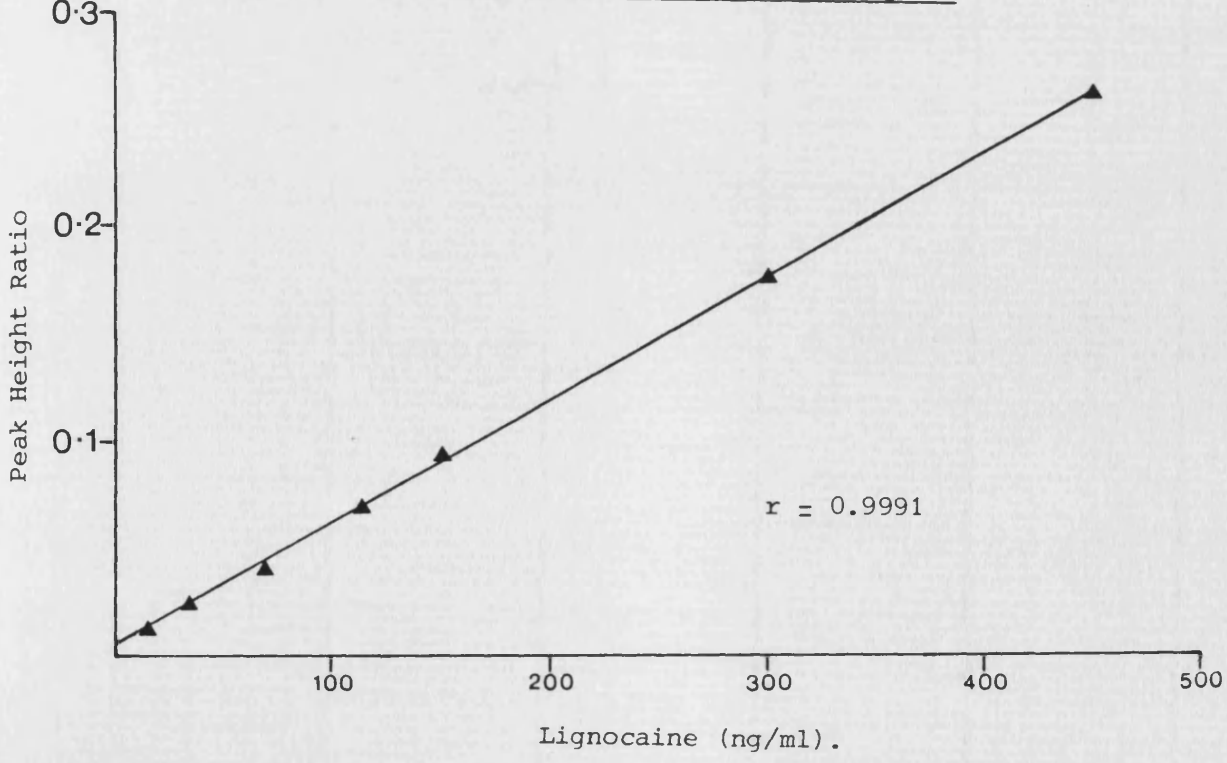


Figure 2.7.5

3-hydroxy-Lignocaine Standard Curve.

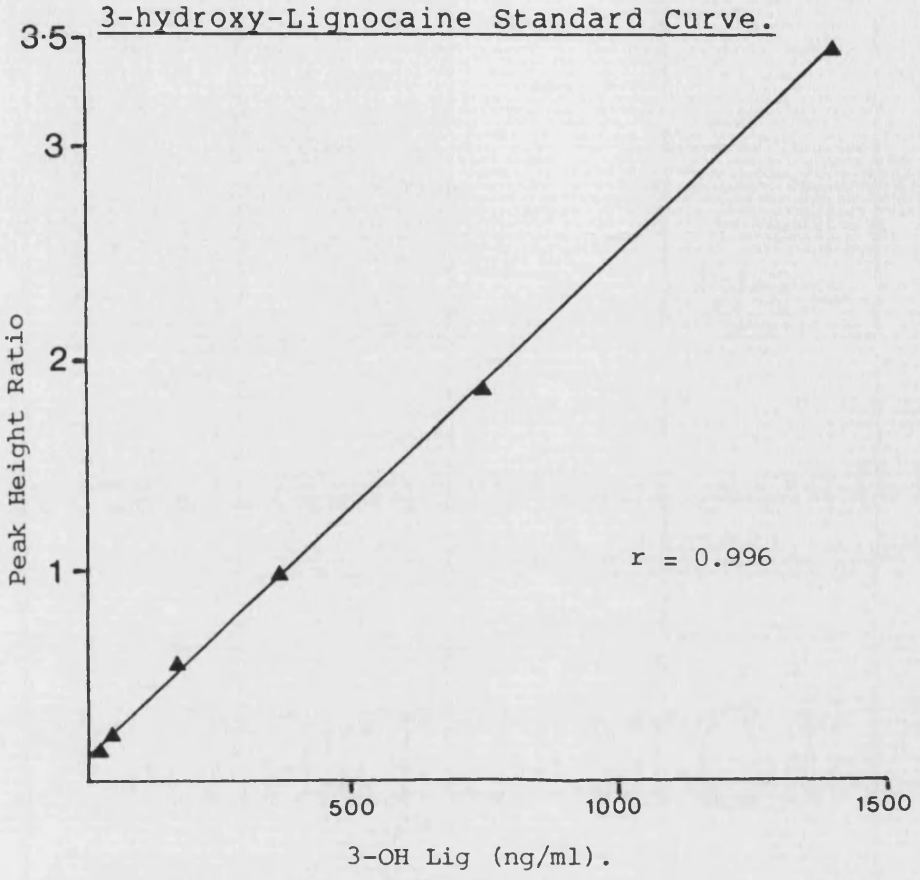


Figure 2.7.6

3-hydroxy-Monoethylglycine xylidide standard curve.

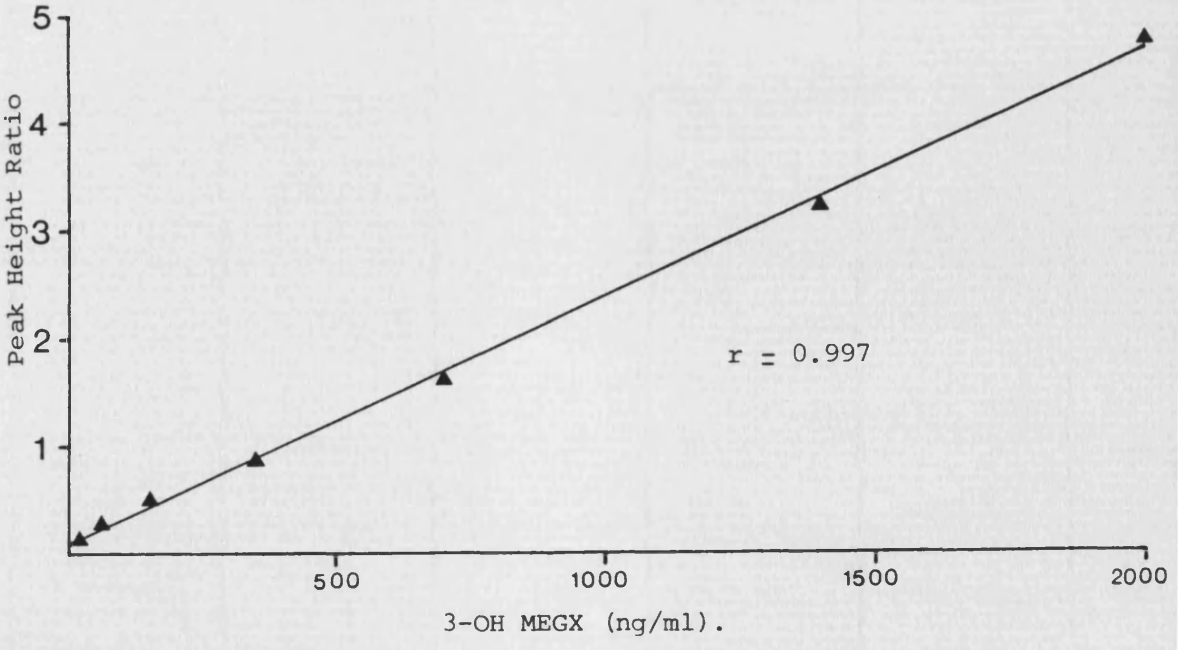


Figure 2.7.7

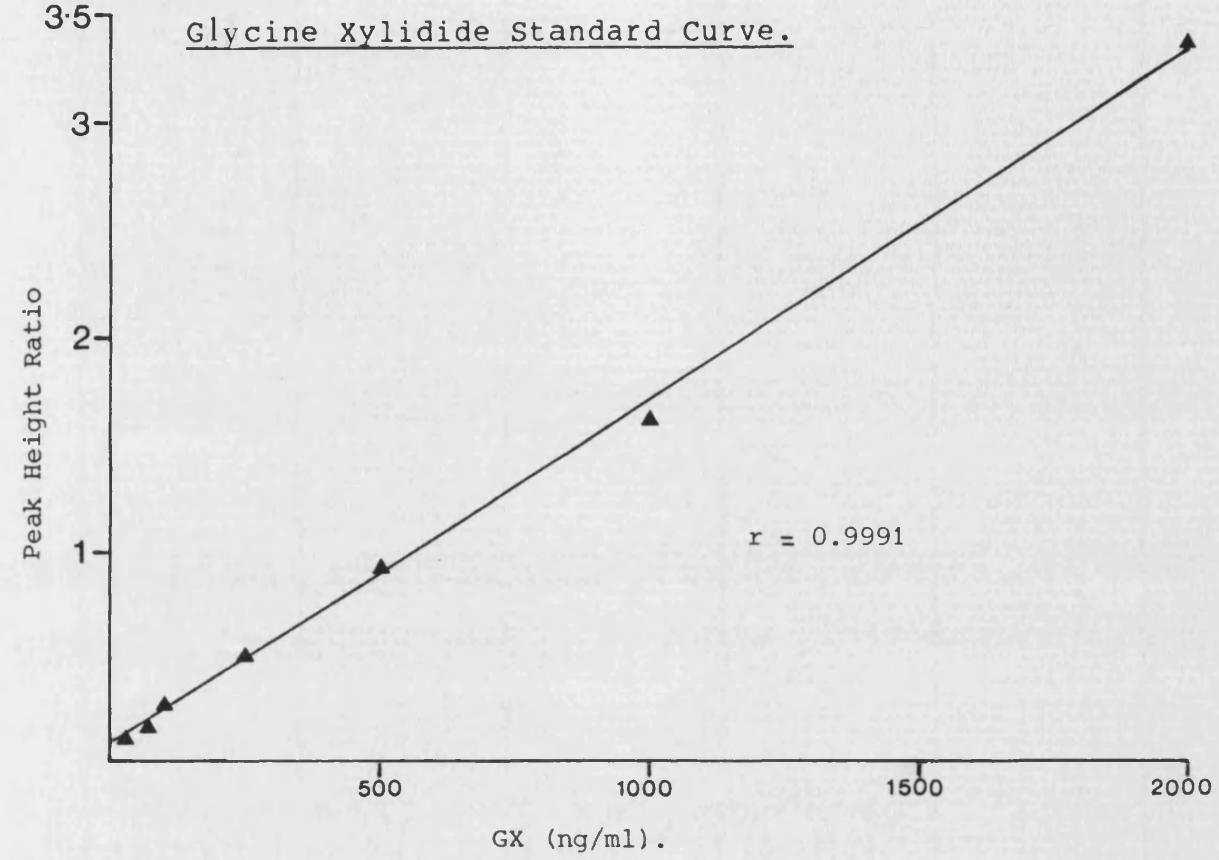
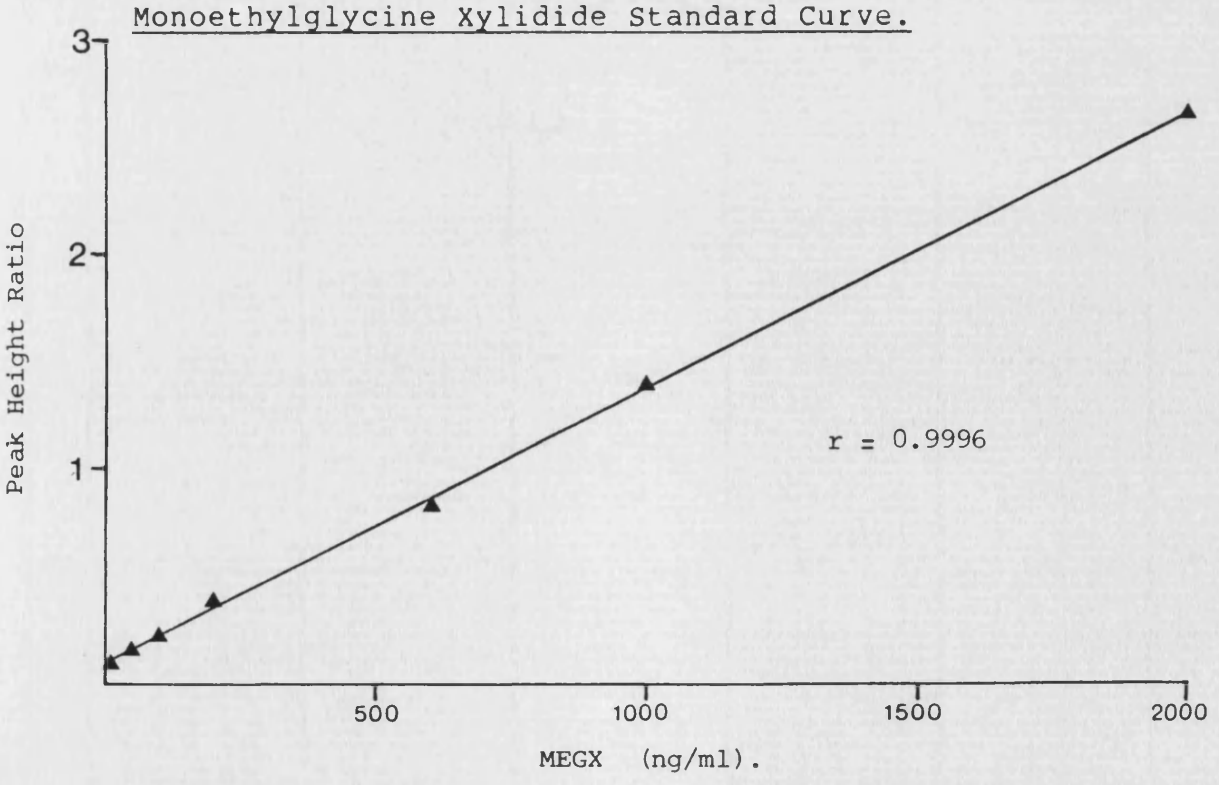


Figure 2.7.8



8) EXPERIMENTAL PROTOCOLS.

(a) Lignocaine extraction ratio and metabolism during changes in the hepatic artery to portal vein flow.

These experiments were carried out on male Wistar rats (University of Bath CFHB strain). Perfusion of the liver set up as a described in sections 4, 5 and 6. During these experiments total liver blood flow remained at 10 ml/min. To examine the effect of changes in the HA:PV flow ratio on the extraction ratio of lignocaine, hepatic artery flow contributions of 7.5, 5, 2.5, 1 and 0 ml/min were used with the corresponding portal vein flow rate to keep total liver blood flow at 10 ml/min. Due to problems with high perfusion pressures encountered at arterial flow rates greater than 5 ml/min, the effect of changes in the HA:PV flow ratio on metabolism were only assessed at arterial flow rates of 5, 2.5 and 0 ml/min.

Lignocaine was added to the perfusion medium reservoir to give a final concentration of 4 ug/ml. Each rat liver was perfused for 60 minutes with liver output samples being collected 0-10, 10-20, 20-30, 30-45 and 45-60 minutes after perfusion equil-

ibrium had been reached. A record of the volume of the perfusate output during each time period was made to ensure that no leaks were occurring in the preparation. Lignocaine and its metabolite concentrations in both liver input and output perfusion medium were determined using the HPLC methods outlined in section 7.

(b) Changes in HA:PV flow ratio on further metabolism of lignocaine metabolites.

This series of experiments was again performed on the rat liver preparation as described in sections 4, 5 and 6. HA:PV flow ratios of 5:5 ml/min and 0:10 ml/min were used. All four metabolites were infused into the liver in concentrations equimolar to 4 ug/ml lignocaine infusions. Rat livers were initially perfused at a HA:PV flow ratio of 5:5 ml/min. Two perfusate samples, 0-10 and 10-20 minute, were taken. The hepatic artery was then clamped and the portal vein flow increased to 10 ml/min. After a ten minute period two consecutive ten minute perfusate samples were taken, giving two perfusate samples for each flow ratio taken over a period of twenty minutes. Metabolite concentrations in both perfusion input and outputs were measured using the HPLC methods described in section 7.

(c) Administration of vasoactive agents on the liver vasculature.

These experiments were carried out on the rat liver preparation as described in sections 4, 5 and 6. An HA:PV flow ratio of 2.5:7.5 ml/min was used in all experiments. Initial experiments had shown that single injections of potential vasoactive agents into either the portal vein or hepatic artery supply were ineffective at eliciting any changes in perfusion pressure. Thus a slow infusion pump apparatus as described in section 4 was employed to administer continuous doses of vasoactive agents. The experiments concentrated on measuring adrenoreceptor populations and the folling protocols were used to measure α and β-adrenoreceptors within the liver vasculature:-

(i) α-adrenoreceptors.

For these experiments it was found that adrenaline and noradrenaline produced vasoconstriction as measured by an increase in perfusion pressure in both the hepatic artery and portal vein, (figures, 2.8.1 and 2.8.2). The increase in pressure in both vessels was dose related and gave sigmoidal log dose response curves. The sigmoidal nature of the log dose

Figure 2.8.1. An Example of Pressure Readings in the Hepatic Artery and Portal Vein During Portal Vein Infusions of Adrenaline, (Similar Responses were Obtained for norAdrenaline).

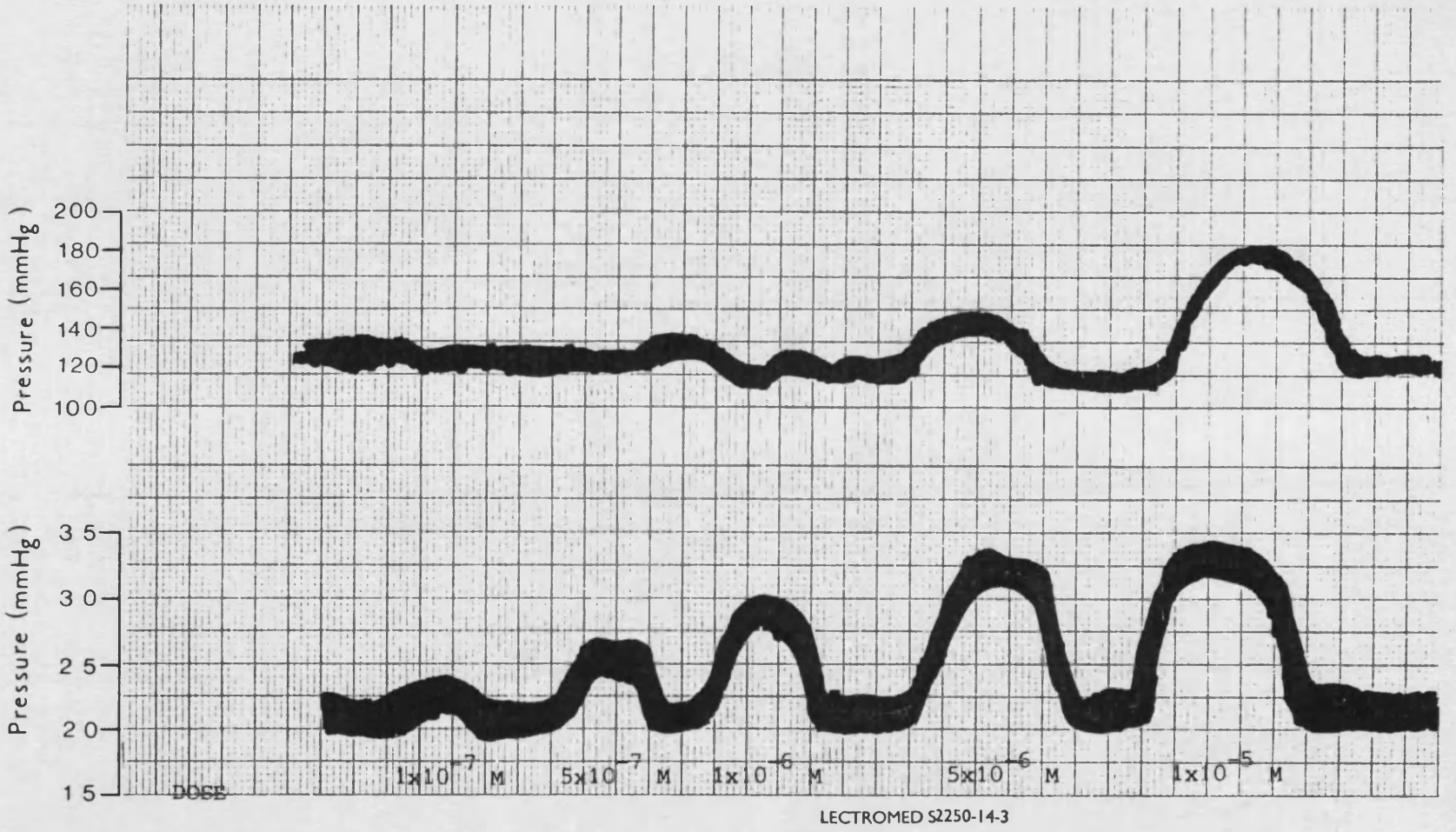
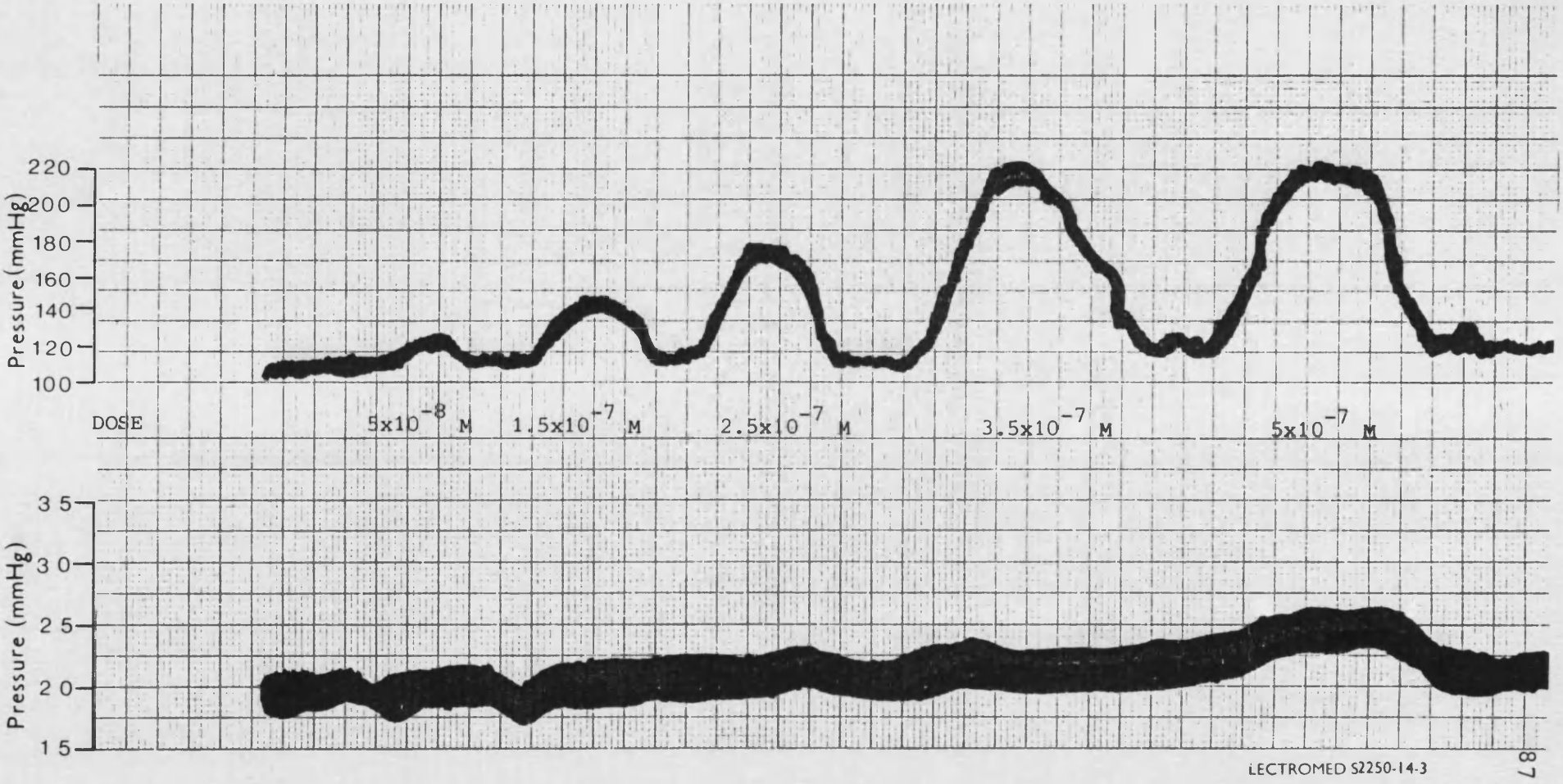


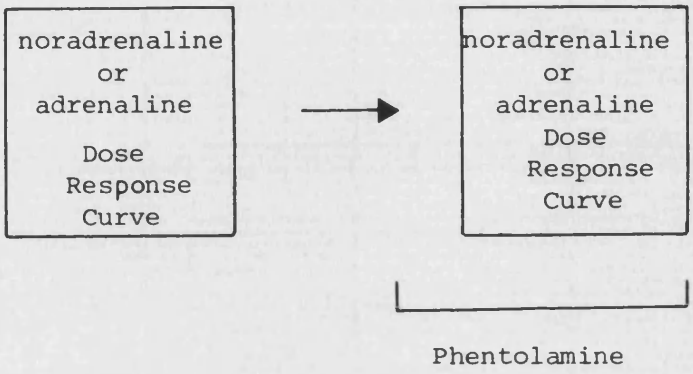
Figure 2.8.2. An Example of Pressure Readings in the Hepatic Artery and Portal Vein During Hepatic Artery Infusions of Adrenaline, (Similar Responses were Obtained for norAdrenaline).



response curves for adrenaline and noradrenaline allowed the calculation of a ED50% for each curve. The ED50% being defined as the dose of adrenaline or noradrenaline required to produce 50% of the maximum increase in pressure. As both adrenaline and noradrenaline infusions produced increases in perfusion pressure in each vessel they may be regarded as vasoconstrictors of the liver vasculature. In order to assess the effect of α-adrenoreceptor antagonists on pressure increases due to adrenaline or noradrenaline infusions, a dose response curve to the vasoconstictor, (adrenaline or noradrenaline) was first constructed. Either adrenaline or noradrenaline being tested in the hepatic artery or the portal vein. After a return to base line pressure in the test vessel an α-adrenoreceptor antagonist was infused into the vessel and a second dose response curve for the vasoconstrictor was contructed. Any change in the ED50% of this second curve as compared to the the first would reflect the degree of antagonism by the α-adrenoreceptor antagonist.

figure 2.8.3

A Diagram of the Protocol used to Measure α-adrenoreceptor Activity.



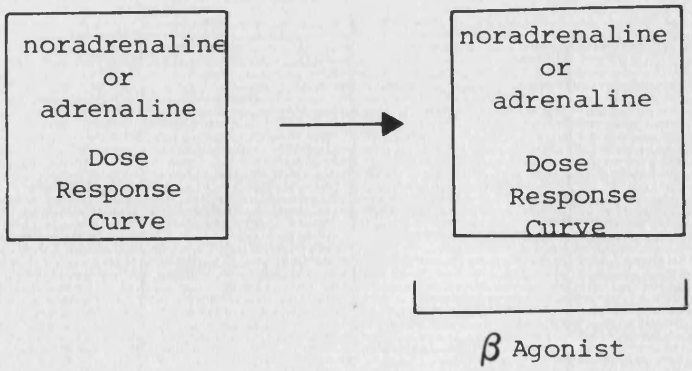
(ii) β-adrenoreceptors

It proved impossible to show any direct dilatory β-adrenoreceptor response as shown by a decrease in perfusion pressure in the hepatic artery or vein during infusion of β agonists into the vessels. In order for dilatory effects to be seen, tone had to be induced in the vessels of the liver. This could be achieved using infusions of adrenaline or noradrenaline but a constant tone could not be maintained with these

agents. Variations in ion concentration of the media could also induce tone but tended to lead to haemolysis of the red blood cells. As an alternative method, adrenaline or noradrenaline dose response curves were produced in the test vessel. A β adrenoreceptor agonist was then infused . A second adrenaline or noradrenaline dose response curve was then produced during β agonist infusion. Any changes in log ED50% between these two curves could be used as an indirect measure of β-adrenoreceptor mediated dilation.

Figure 2.8.4

A Diagram of the Protocol used to Measure β-adrenoreceptor Activity.

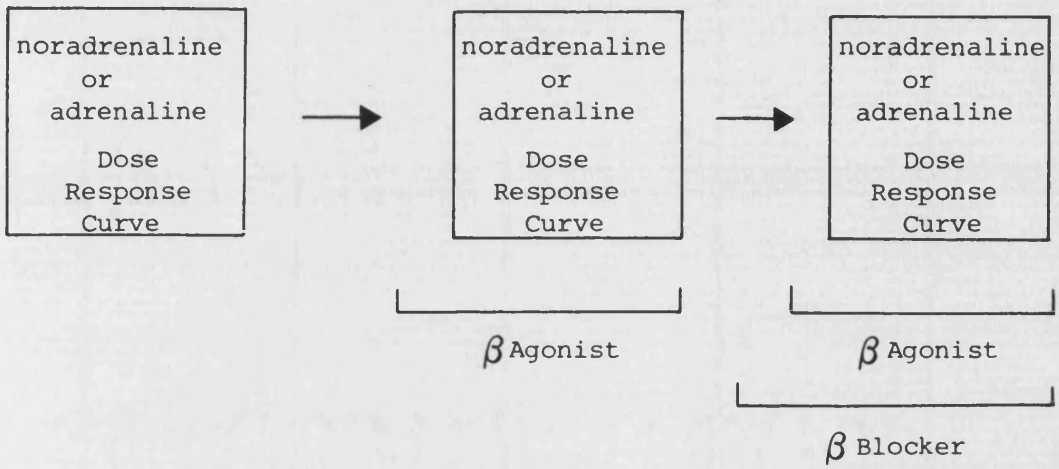


(iii) β_1 and β_2 -adrenoreceptors

To ensure that any changes in log ED50% of adrenaline or noradrenaline, seen during β-adrenoreceptor agonist infusion, were due to β agonism and not α-adrenoreceptor antagonism, β-adrenoreceptor blocking drugs were used to test the specificity of any β-adrenoreceptor mediated response. β blockers also allowed the classification of the β-adrenoreceptors in to β_1 and β_2 subtypes. β-adrenoreceptor activity was measured by the shift in log ED50% of adrenaline or noradrenaline as outlined in section (ii). After a five minute rest from vasoactive stimulation the test vessel was perfused with a β-adrenoreceptor blocking drug by slow infusion into the perfusion medium. After a 5 minute period of β blocker perfusion, a β-adrenoreceptor agonist was co-infused with the β blocker. A third adrenaline or noradrenaline dose response curve was then produced during combined β blocker and agonist infusion. Any shift of log ED50% in this third constrictor from that obtained in the absence of β-adrenoreceptor blockade was taken as a measure of β-adrenoreceptor blockade. This rather complex method was used to test the specificity of β-adrenoreceptor dilation and to characterise the β-adrenoreceptor population in the liver vasculature.

Figure 2.8.5

A Diagram of the Protocol used to Measure β_1 and β_2 -adrenoreceptor Activity.



(iv) Portal vein - Hepatic artery vasculature interactions.

It was noted that in isolated rat liver perfusions, hepatic artery infusions of adrenaline and noradrenaline only occasionally produced increases in portal vein perfusion pressure. However, portal vein infusions of adrenaline or noradrenaline did produce a dose dependent increase in hepatic artery perfusion pressure. To investigate this interaction isolated rat

livers were perfused at an HA:PV ratio of 2.5:7.5 ml per minute using the standard procedures outlined previously. The following protocol was then used:-

1) Standard log dose response curves for noradrenaline were obtained in both the hepatic artery and the portal vein. A third standard log dose response curve was obtained for increases in hepatic artery perfusion pressure during portal vein infusions of noradrenaline.

2) 1×10^{-6}M phentolamine was then infused into the portal vein and all three log dose response curves re-established.

3) Portal vein phentolamine was then removed and selected doses of noradrenaline were used to test when the effects of the phentolamine had disappeared.

4) 1×10^{-6}M phentolamine was then infused into the hepatic artery and all three log dose response curves re-established.

(d) Perfusion pressure and lignocaine metabolism.

These experiments were again carried out on perfused rat livers prepared as described in sections 4, 5 and 6. The perfusion medium contained lignocaine at a concentration of 4 ug/ml and the hepatic

artery portal vein flow ratio during the perfusion was 2.5:7.5 ml/min. After an initial twenty minute equilibration period the output perfusate was collected for a period of five minutes. This sample was used to test the extraction ratio and metabolism of lignocaine under normal pressure conditions. Adrenaline and noradrenaline were used as vasoconstrictors in order to raise the perfusion pressure these experiments. After the initial output sample had been taken, dose response curves were obtained for either constrictor, (adrenaline or noradrenaline), in both the hepatic artery and the portal vein. A dose of constrictor giving a 70% to 80% maximal increase in pressure was chosen for both the hepatic artery (HA_{dose}) and the portal vein (PV_{dose}). The liver was then allowed a five minute recovery period before the following schedule was carried out:-

(i) The artery was infused with HA_{dose} of the constrictor. When a constant increased pressure was attained the liver was perfused for a further three minutes before a five minute perfusate sample was taken.

(ii) The infusion of the constrictor was stopped and the arterial pressure allowed to return to normal.

(iii) Five minutes after baseline pressure had been attained and maintained, 5 x 10^{-6}M phentolamine was infused into the hepatic artery supply. Three minutes after the start of phentolamine infusion a five minute perfusate sample was taken.

(iii) The hepatic artery was then co-administered with phentolamine and HA_{dose} of constrictor. No pressure increase was seen in any experiment as the phentolamine blocked vasoconstriction. After three minutes of co-administration, another five minute output sample was taken.

(iv) Constrictor and phentolamine infusions were removed and a five minute recovery period allowed.

(v) PV_{dose} of the constrictor was infused into the portal vein. After a constant increased portal vein pressure had been attained the liver was perfused for a further three minutes before a five minute perfusate sample was taken.

(vi) The constrictor infusion was removed and after portal pressure had returned to normal a five minute recovery period was allowed.

(vii) 1 x 10^{-5}M phentolamine was infused into the portal vein and after three minutes a five minute perfusate sample was taken.

(viii) The portal vein was then co-infused

with both PV_{dose} of the constrictor and phentolamine after a three minute perfusion period a final five minute perfusate sample was taken.

(ix) Samples were then measured for lignocaine and metabolite concentrations by the HPLC methods in section 7.

(e) Effect of hydralazine on systolic blood pressure.

Male Wistar rats weighing 400-450g were used and their systolic blood pressure measured by tail artery measurements. The apparatus used consisted of a tail pressure pneumatic pulse transducer (Narco, Texas, USA). A type CR 650S JJ recorder, (JJ instruments, Southampton), was used to record the traces. The rats were restrained in a rat restrainer with a Mark IV temperature controller, (Narco, Texas, USA), set at $37^{o}C$.

Rats were first acclimatised to the restrainer and after they had given three consecutively normal (approximately 120 mmHg) pressure readings they were used experimentally. To measure blood pressure in this system rats were placed in the restrainer and the cuff placed over the tail approximately three quarters of the way from the tip. The pulse transducer was then

attached over a tail artery with Sellotape so that a pulse could be seen on the recorder. The recorder trace measured the pressure inside the cuff and superimposed the pulses on this trace. The cuff was then inflated and automatically deflated. As the cuff pressure increased so the height of the pen above baseline on the recorder also increased. The height at which a pulse was lost represented the pressure at which internal cuff pressure equaled arterial blood pressure. On deflation, the height of the trace at where the pulse was regained also represented arterial blood pressure. The average of these two heights gives an accurate average systolic pressure measurement.

To test the effect of hydralazine on systolic blood pressure, two groups of animals were used. One group was given an oral dose of water at time zero and represented a control group. The second group was given hydralazine hydrochloride, 1 mg/kg, in water at time zero. The blood pressures of both groups were measured at 15, 30, 45, 60, 90, 120, 180 minutes after dosing.

(f) Lignocaine clearance after hydralazine treatment.

Male Wistar rats, Bath University CFHB strain, weighing between 400-450g were used in these experiments. Rats were divided into four groups; two control and two experimental. Control groups received water orally, and the other two groups received hydralazine hydrochloride, 1 mg/kg, dissolved in water, orally. The animals were then left for 40 minutes in which time they had free access to water and food.

To one control group and to one experimental group, lignocaine hydrochloride, 20 mg/kg, in water, was given orally. To the second control and experimental group, lignocaine hydrochloride, 2.5 mg/kg, was given as an I.V. bolus injection. The lignocaine was dissolved in isotonic saline and given by tail vein injection.

At 5, 10, 15, 20, 30, 45, 60, 90, 120 and 180 minutes after injection blood samples were taken by cardiac puncture under light anaesthesia. One blood sample per rat was taken; then the animal was killed by cervical dislocation. Blood samples were collected in heparinised tubes and spun at 2000g, (IEC Centra-7, Damon, Bedford, UK), to separate the plasma. Plasma samples were decanted into glass vials,

which were immediately frozen at -20°C. 1 ml plasma aliquots were diluted with distilled water to a volume of 5 ml and the lignocaine concentration measured by the HPLC method of section 7.

(g) Changes in liver blood flow after hydralazine treatment.

Assessment of liver blood flow was carried out using the dye indocyanine green and a modification of the method used by Iga et al. (1980). This dye is removed from the blood stream only by the liver and does not undergo enterohepatic circulation. Its disappearance from the circulation is dependent on liver blood flow and it may be used as an index to compare liver blood flow.

Male Wistar rats, Bath University CFHB strain, were used in these experiments. Large animals weighing between 500-550g were used to minimise haemodilution effects. The animals were divided into two groups; the control group received water orally, the other hydralazine hydrochloride in water, 1 mg/kg, given orally. The animals were then left for twenty minutes after which both groups underwent the foll-

owing procedures.

Animals were anaesthetised with pentabarbitone, 75 mg/kg (6% Sagatal, May and Baker, Dagenham, England). The animals were then given heparin dissolved in isotonic saline, 1000 units, by intraperitoneal injection and placed on a heated surgical board kept at 25°C. The left carotid artery was cannulated using a heparinised 20 gauge catheter placement unit, (Quick Cath, Dupont Travenol, Laboratories, Ireland). A water-tight rubber membrane was then placed over the outflow of the cannula. Blood samples from the cannula could thus be withdrawn with minimum disturbance and blood loss. A second 20 gauge heparinised catheter placement unit, (Quick Cath, Dupont Travenol Laboratories, Ireland), was used to place a cannula in the right jugular vein. The animals were left for 5 minutes. A control 0.3 ml blood sample was then taken from the carotid artery cannula. This was followed one minute later by a bolus injection of indocyanine green, dissolved in saline, 6uM/kg given via the jugular vein cannula. The dye was flushed into the circulation with a further injection of 0.5 ml of isotonic saline. The jugular vein cannula was then sealed. The time of dye injection was taken as time zero and 0.25 ml blood samples were then taken at

1, 3, 5, 7, 10, 15, 20, 30, 45, 60, 75 and 90 minutes after time zero. These samples were spun in a high speed, type 54414, Eppendorf centrifuge, (Eppendorf, Hamburg, West Germany), for two minutes to separate the plasma. A 0.1 ml aliquots of plasma were taken and mixed with 0.9 ml of deionised water. The absorbance, at 800nm, of these solutions was then measured ten minutes later. This assay was carried out on a Pye spectrophotometer, (Pye Unicam, PU 8610, Cambridge, England) at 25^{o}C using the initial control plasma sample as a blank. All samples were measured between 15 and 20 minutes after removal from the animal.

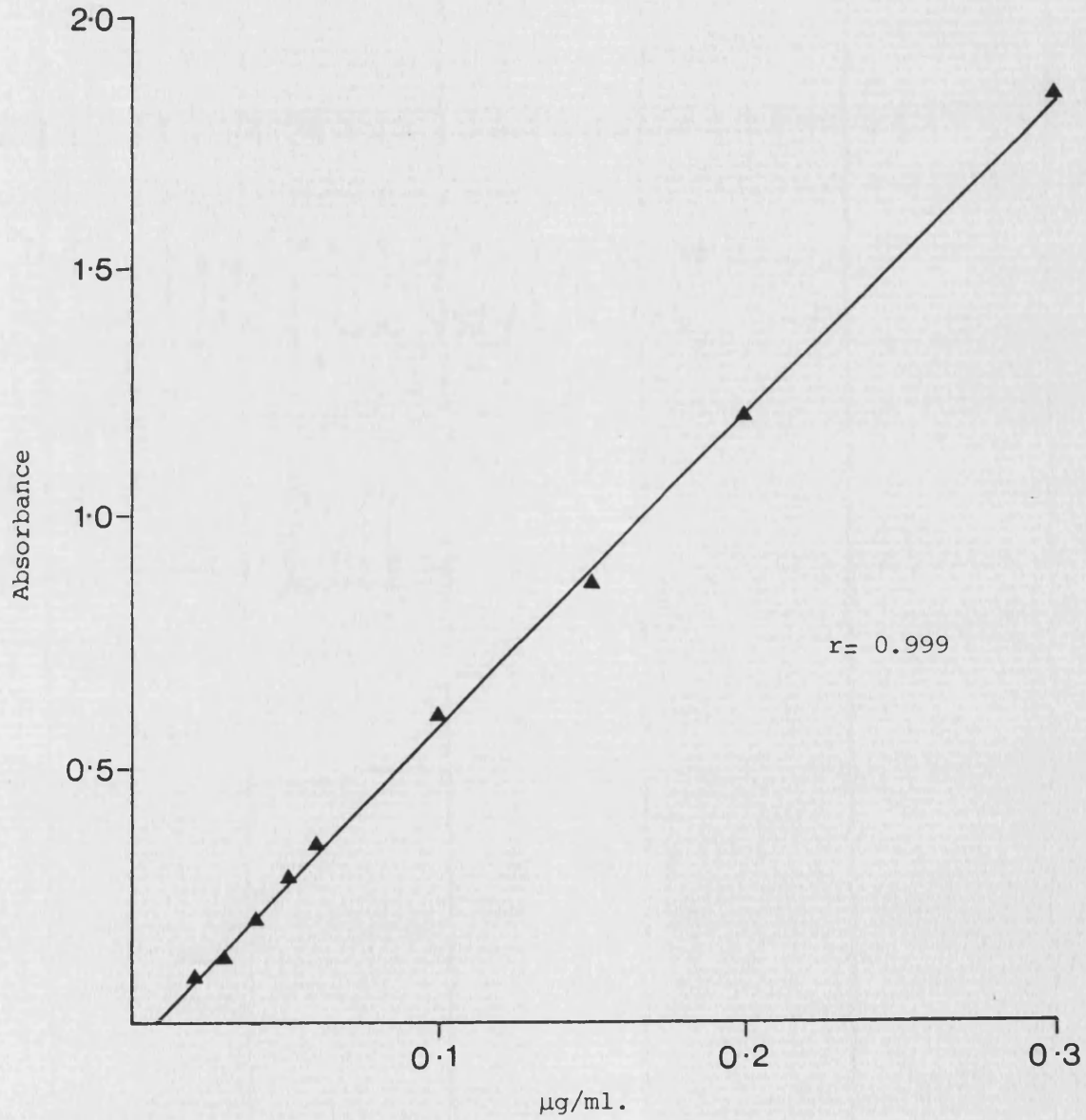
A standard curve of absorbance against concentration was prepared. This was constructed using 0.9 ml samples of known dye concentration independently diluted in deionised water to which 0.1 ml of pooled blank rat plasma was added. These samples were measured by the same system as the plasma samples. The standard curve (fig. 2.8.6), produced had a linear correlation coefficient of 0.999.

(h) Lignocaine tissue binding after hydralazine pretreatment.

Male Wistar albino rats, Bath University CFHB strain, weighing between 400-450g were used. The

Figure 2.8.6

Indocyanine Green Standard Curve.



animals were divided into two groups. A control group received hydralazine hydrochloride dissolved in water, 1 mg/kg, orally. The animals were then left for 40 minutes before all were given lignocaine dissolved in saline, 25 mg/kg, by a bolus tail vein injection. The lignocaine solution used contained ^{14}C-labelled lignocaine, (New England Nuclear, Dupont, Southampton, England.), at an activity of 2 uCi/ml. Immediately following the lignocaine injections 1000 units of heparin were administered to each rat by intraperitoneal injection.

Two minutes after the lignocaine injection rats were bled under light ether anaesthesia by cardiac puncture. The blood was collected in heparinised tubes and spun down at 2000g (IEC Centra-7, Damon, Bedford, UK.), for 10 minutes to separate the plasma. Immediately after bleeding the animals were killed by cervical dislocation. The chest cavity was then opened by two incisions through the rib cage. A cannula was then placed in the inferior vena cava using a 14 gauge catheter placement unit, (Venflow Viggo, Helsingborg, Sweden). As much remaining blood as possible was removed from the circulation. The abdomen was opened and the renal arteries and portal vein severed. 50 ml of Krebs buffer was then passed

through the vena cava cannula to remove as much residual blood as possible from the kidneys and liver.

The heart, lungs, kidneys and liver were all removed, damped dry and weighed. A sample of each organ, of known weight was taken and finely chopped using scissors. The tissue was washed several times with Krebs buffer to remove any blood still remaining. The samples were then damped dry and mixed with 5 ml. of Krebs buffer. The tissues were homogenised using a TRI R type 1K41 homogeniser, (TRI R Instruments, New York, USA).

0.5 ml of each homogenate and 0.5 ml of plasma were then mixed with 10 ml of Optiphase "Safe" scintillation fluid, (Fisons, Loughborough, UK) in glass counting vials. The radioactivity of each sample was measured using an LKB 1215 Liquid Scintillation counter linked to a KODE Model 43 printer, (Wallac, Finland), using the appropriate quenching corrections.

(i) Lignocaine binding to red blood cells and plasma proteins during incubation with hydralazine.

In this experiment whole rat blood was obtained from male Wistar rats, CFHB strain, by cardiac puncture, under light ether anaesthesia. The blood was collected in heparinised tubes and spun down at 2000g, (IEC Centra-7, Damon, Bedford, UK.), for ten minutes, to separate out the red blood cells. Nine tubes were then set up each containing 2 ml of plasma and 2 ml of packed red blood cells.

Using a stock solution of lignocaine hydrochloride, 0.1 mg/kg, having an activity of 2.2 uCi/ml, (New England Nuclear, Dupont, Southampton, U.K.). Tubes containing 7 ug/ml, 2.5 ug/ml, 0.7 ug/ml and 0.25ug/ml, final lignocaine concentration were made up, (two tubes at each concentration). To one tube of each dilution hydralazine to a final concentration of 1×10^{-4} M was added. To the other four tubes the same volume of Krebs buffer was added. A ninth tube contained no lignocaine and no hydralazine and was used to assess the background activity of the solutions used. All tubes were then oxygenated by bubbling 95% Oxygen 5% Carbon Dioxide gas through the solutions. All were then incubated at $37^{\circ}C$ for 1

hour in a shaking water bath.

After this incubation period the solutions were spun down at 2000g (IEC Centra-7, Damon, Bedford, UK), for ten minutes to separate the red blood cells. 1 ml of plasma was removed and filtered using Amicon MPS 1 YMT 14mm filters, placed in MPS-1 filtration units, (Amicon, Gloucester, England). The plasma samples were spun at 2000g for twenty minutes in a Heraceus Christ labfuge 1, (Heraceus Christ GmbH Germany). 0.2 ml of the protein free filtrate, 0.1 ml of red blood cells and 0.7 ml of whole plasma for each lignocaine dilution were placed in glass counting vials containing 10 ml. of scintillation fluid, (Optiphase "Safe", Fisons, Loughborough, UK). The activity of each sample was then measured on a LKB 1215 Liquid Scintillation Counter linked to a KODE model 43 printer, (Wallac, Finland). Using this method the binding of lignocaine to plasma was determined from the difference between whole plasma activity and protein free filtrate activity. Binding to red blood cells was determined directly.

(j) Effects of Hydralazine on the isolated perfused heart.

Isolated hearts were perfused retrogradely. Male Wistar rats, Bath University CFHB strain, weighing between 200-250g were killed by cervical dislocation. The heart was removed and chilled in ice cold Krebs solution to allow easier manipulation. The heart was perfused with a Krebs buffer, (Appendix 2). The Krebs buffer used in the isolated heart experiments differed slightly from that used in liver perfusions. This Krebs buffer was only used on isolated heart preparations and will be denoted by Krebs buffer (Heart). The isolated hearts were perfused at a flow rate of 10ml/min using a peristaltic pump, (Watson Marlow, Falmouth, England). Perfusion pressure was monitored using a Bell Howell type 4-422-0001 pressure transducer, (Hertfordshire, England), attached to a Gould 2400 S recorder, (Gould instruments, Ohio, USA). An E.C.G. trace was also obtained using needle electrodes placed on the right atrium and left ventrical; the trace being recorded on a Electromed type MX 216, 2 channel recorder, (Ormed, Hertfordshire, England). The trace was also used to determine heart rate.

After the preparation parameters had stabilised the heart was left for twenty minutes to

ensure equilibration. A measurement of heart rate was made. Then Krebs buffer (Heart) containing 10^{-4}M hydralazine was infused for ten minutes, after which heart rate measurements were again taken. Krebs buffer (Heart), containing no hydralazine was then perfused After a 15 minute period of perfusion heart rate readings were taken. Then Krebs buffer containing 10^{-3}M hydralazine was infused and after ten minutes further readings were taken.

CHAPTER 3

RESULTS AND DISCUSSION

1) THE EFFECT OF ALTERATIONS IN THE HA:PV FLOW RATIO ON THE METABOLISM OF LIGNOCAINE.

(a) Lignocaine extraction ratio.

The extraction ratio of lignocaine was calculated using the formula given in equation 3.1.1

$$E = \left(\frac{C_{IN} - C_{OUT}}{C_{IN}} \right) \times 100 \qquad \text{Equation 3.1.1}$$

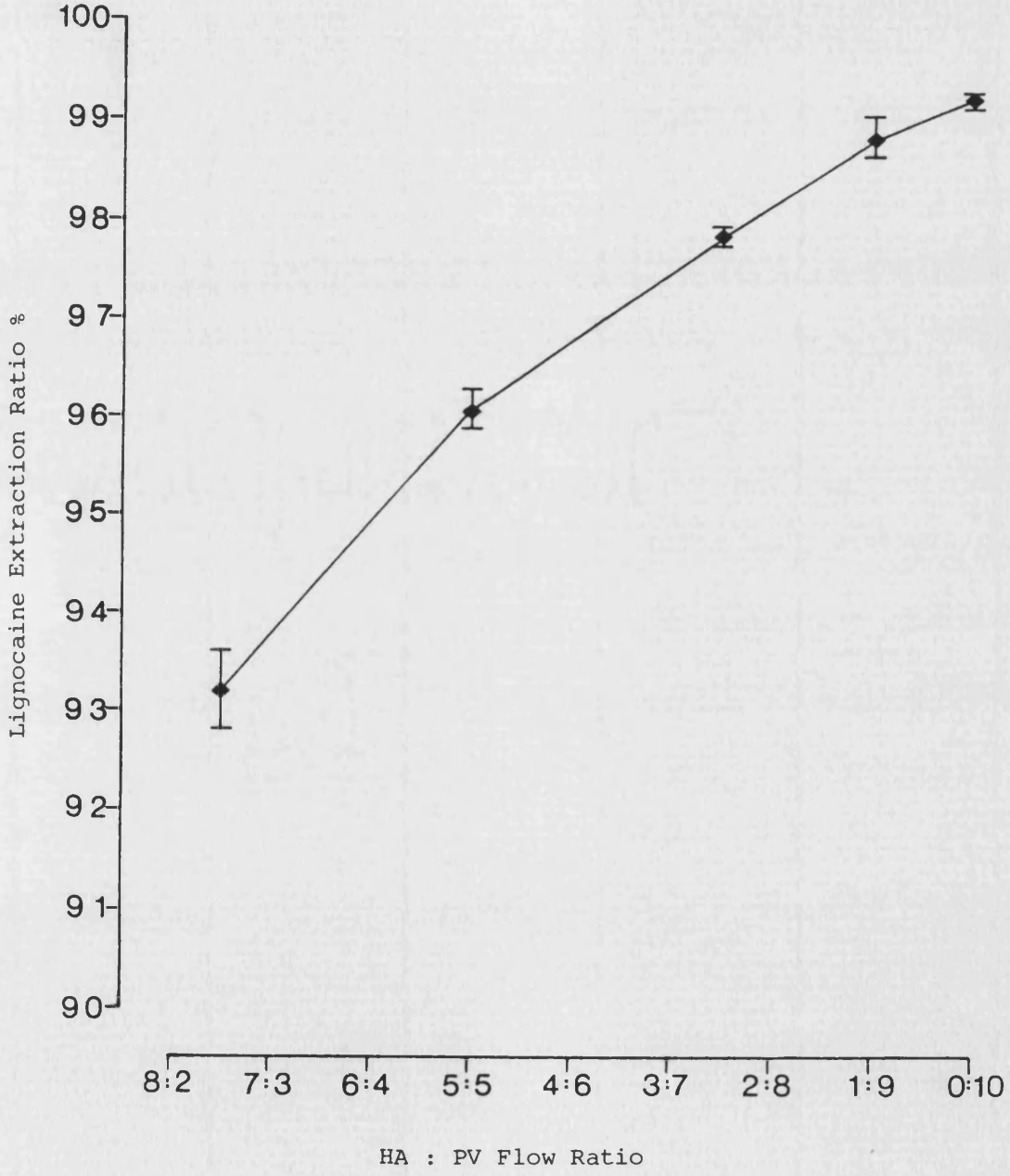
E is the extraction ratio.

C_{IN} is the input lignocaine concentration in both the hepatic artery and portal vein.

C_{OUT} is the output lignocaine concentration.

Figure (3.1.1) shows the extraction ratio of lignocaine during different HA:PV flow ratios. The extraction ratio of the drug increases as the portal vein flow contribution is raised. These results are similar to those obtained by Ahmad (1983) in which he also showed that as portal vein flow contribution increased, so extraction of lignocaine by the liver became more efficient. Several possible explan-

Figure 3.1.1. The Effect of Variation in the HA:PV Flow Ratio on the Extraction Ratio of Lignocaine



Points represent the mean ± sem.

See Appendix 3 for data and n values.

ations may be put forward to account for these results.

i) Shunting.

One possible explanation for these results is the phenomenon of shunting, which occurs when blood is diverted or shunted through non-metabolic channels within the liver. Any drug in shunted blood will not come into contact with the metabolising enzymes and will appear in the venous system. The diversion of drug will result in an increase in venous concentration and a decrease in hepatic extraction ratio. Although shunting occurs in diseased livers, (Gross et al., 1975, Groszmann et al., 1977), it also occurs to a small degree in normal rat livers (Wood et al., 1979), and human liver, (Groszmann et al., 1977). Ahmad (1982), found that shunting in the isolated perfused rat liver was measurabley greater when the liver was perfused through the hepatic artery than when it was perfused by the portal vein. Shunting would seem to offer one possible explanation for the increase in extraction ratio seen with increases in portal vein flow contribution as at high hepatic artery flow rates shunting is greatest.

ii) Separate enzyme environments between the hepatic artery and portal vein.

An alternative explanation for the results seen during variation in HA:PV flow ratio is that the hepatic artery and the portal vein do not pass through the liver via the same sinusoids and/or that those sinusoids which receive both portal vein and hepatic artery blood do so in proportions which differ from the input HA:PV flow ratio. These separate, or more highly perfused portal vein or hepatic artery channels, may exhibit heterogeneity with respect to their metabolising environments. These two phenomena may result in blood passing into the liver via the portal vein being exposed to a different metabolic environment to that seen by hepatic artery blood. An increase in the proportion of blood flowing into the portal vein would increase the quantity of blood exposed to the postulated portal environment and may cause changes in the overall liver metabolism of input drugs.

Evidence for separate portal vein and hepatic artery channels through the liver has come from experiments using radioactive transit times (Rees et al., 1964; Hollenberg et al., 1966; Birtch et al., 1967). Experiments by Breedis et al., (1954); Solt et

al., (1977) and Conway et al., (1984) have shown that tumour cells receive predominantly hepatic artery blood but the surrounding non-diseased tissue receives both hepatic arterial and portal venous blood. More highly perfused hepatic artery channels may exist in the normal liver. The results indicate that complete mixing of hepatic artery and portal vein blood cannot be assumed. Flexibility of the liver blood supply to sinusoids has been shown by Wakin et al., (1942); Bloch (1955), and McCuskey (1966) who all showed the presence of "static sinusoids" which can be switched in and out of the general circulation using input and output sphincters. Wakin et al. (1942) calculated that at any one time 75% of the liver sinusoids may be in this inactive state. Elias et al. (1953) and Burkel (1970) in rats have shown the existence of direct arteriole connections to sinusoids which seem to contain predominantly arterial blood. Thus, there is evidence which supports the existence of a degree of HA:PV separation within the liver. Also as outlined in section 4b of the introduction there is a degree of heterogeneity within the liver with respect to Phase I and Phase II drug metabolising enzymes. However no positive link between drug metabolising enzyme heterogeneity and possible separate

hepatic artery or portal vein channels has been shown.

iii) Physical heterogeneity between separate hepatic artery and portal vein channels.

Separate hepatic artery or portal vein channels may show physical differences, for example, with respect to the size of the space of Disse or fenestral diameter. A larger space of Disse would mean that solute molecules would have further to travel before colliding with the parenchymal surface membrane and therefore, extraction of drugs from the plasma would be lower. Also, a decrease in fenestral size would reduce the passage of molecules out of the sinusoid lumen into the space of Disse, also resulting in a decrease in extraction of a drug.

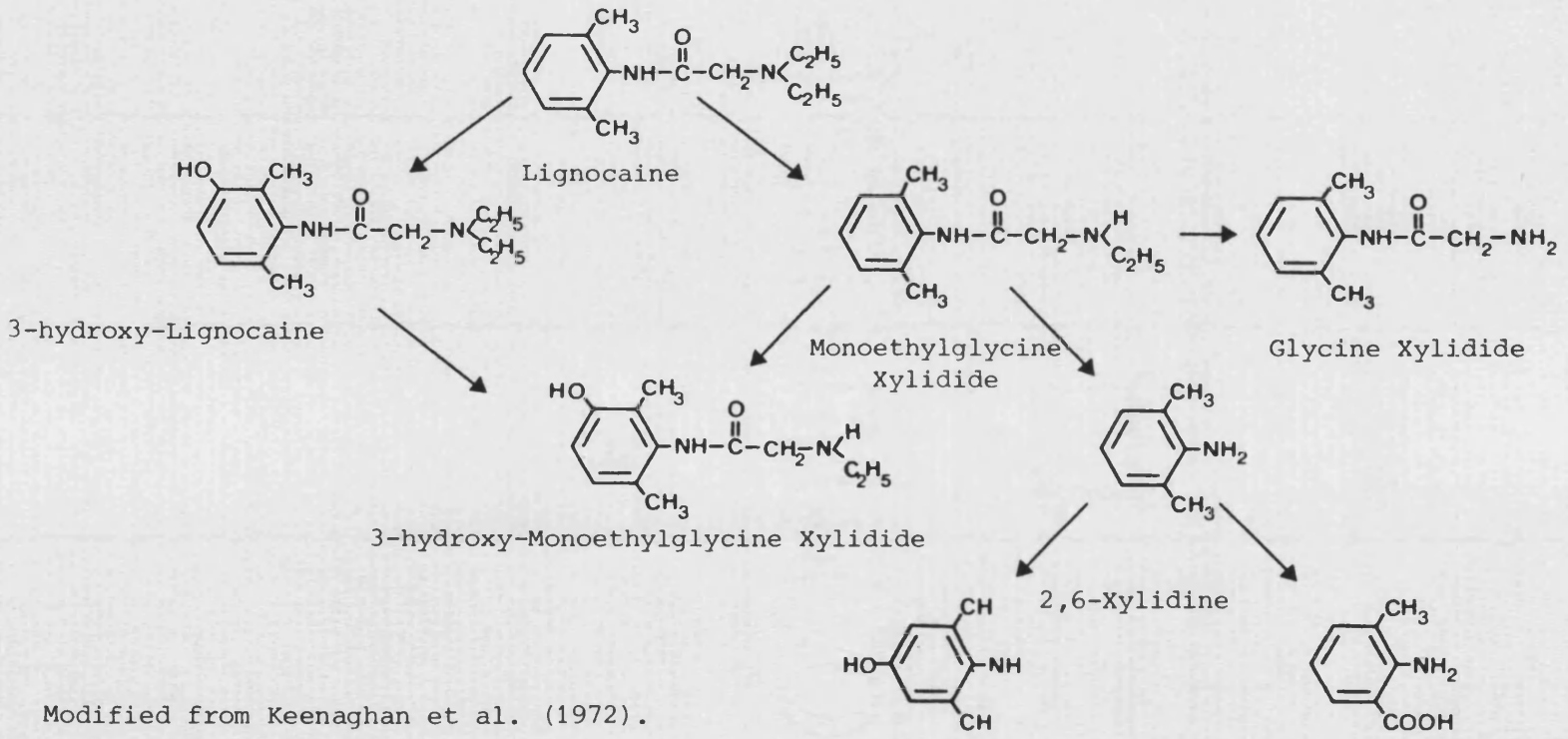
Measurement of the apparent space of Disse may be achieved using the difference between radiolabelled albumin and red blood cell transit times through the liver. Albumin is able to enter the space of Disse but red blood cells can not. Thus, the albumin will be delayed in its passage through the liver by entry into this space. The greater the delay, the larger the space of Disse. However this method is also dependent on fenestral size and number. The greater the porosity of the sinusoid wall the

greater the albumin delay. Experiments by Cohn et al. (1969), showed no significant difference in transit times of albumin and red blood cells between the hepatic artery and portal vein of dogs. However, Ahmad (1982), in rats, did show a greater difference in red blood cell and albumin transit times when the liver was perfused by the hepatic artery as compared with portal vein perfusions. Physical differences such as space of Disse size or fenestral diameter may account for the difference in extraction between the two routes. As flow is proportionally increased through one route the amount of drug exposed a particular environment increases. If the environments differ between the two routes this may be reflected by an overall change in extraction of a drug.

(b) Effect on metabolic profile of Lignocaine.

To investigate possible differences in the metabolic environments of the hepatic artery and the portal vein, the metabolic profile of lignocaine was determined at different flow ratios. Table 3.1.1 shows the results of varying the HA:PV ratio on recovery of lignocaine metabolites either as conjugates or in their free form. Combined total metabolites

Figure 3.1.2. The Known Metabolism of Lignocaine



Modified from Keenaghan et al. (1972).

Table 3.1.1. Total Output Metabolite Concentration Expressed as a Percentage of Input Lignocaine Dose in nM/ml.

FLOW (ml/min) HA : PV	Combined Total Metabolite conc	Glucuronide Conjugates	Sulphate Conjugates	Unconjugated
5 : 5 n = 37 (8 rats)	67.15 ± 1.24 **	31.16 ± 1.88	15.45 ± 1.20 **	17.75 ± 0.51 ***
2.5 : 7.5 n = 35 (7 rats)	60.06 ± 2.24	35.16 ± 1.70	11.95 ± 0.79	12.94 ± 0.51
0 : 10 n = 48 (10 rats)	67.77 ± 1.94 **	33.88 ± 1.37	21.40 ± 1.30 ***	12.54 ± 0.45

Values are Percentages ± Standard errors of the mean.

Significance was Measured from the 2.5 : 7.5 Flow Ratio by the Unrelated "t" test.

Significance

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

represents the sum of the concentrations of all forms of 3-OH MEGX, 3-OH LIG, MEGX, GX and lignocaine in the liver output. These values give an indication of the recovery of input lignocaine dose. For the perfused rat liver the recovery was between 61-68%. This is comparable with the results of Keenaghan et al. (1972) who showed that the metabolites measured in this study should represent 69% of the lignocaine dose when measured in the urine of rats. Recovery of the lignocaine dose is not complete. This is most likely a result of production of metabolites not measured in this study, for example, 4-Hydroxy-2, 6-diethylaniline or 2,6 xylidine which form approximately 14% of the dose recovered in rats, (Keenaghan et al., 1972). Conjugates other than sulphates and glucuronides may also have been produced and again these would not have been measured in the assay. Since it was intended only to compare the metabolic routes of de-ethylation and hydroxylation, it was not necessary to obtain 100% recovery of the lignocaine dose.

The recovery of the lignocaine dose was found to alter at the HA:PV flow ratio 2.5:7.5. This presented a slight problem as only 61-68% of the input dose was recovered and since any changes in metabolic profile were likely to be small, this drop in recovery

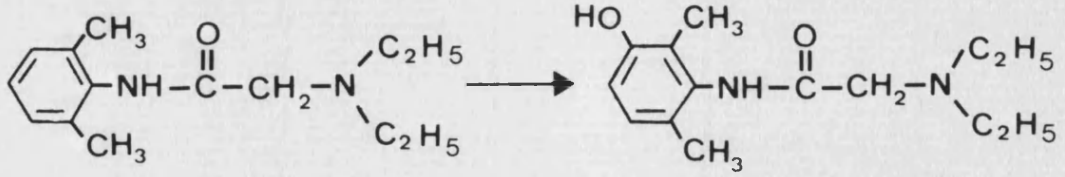
could mean that changes in metabolic profile may be masked by changes in recovery. This situation occurs frequently in pharmacokinetic studies in patients where drug recovery is quite often not consistent. In such experiments metabolite concentrations are expressed as a percentage of the total recovered metabolites. The calculation of the results is outlined in Figure 3.1.3. This way of presenting the results still allows both a comparison of Phase I metabolism using the values for total 3-OH LIG, 3-OH MEGX, MEGX and GX and also Phase II using TOTAL glucuronides and sulphates, as well as allowing assessment of individual metabolite concentrations.

(i) 3-Hydroxy lignocaine (3-OH LIG) formation.

Figure 3.1.4 shows the recovery of the various forms of 3OH Lignocaine. The Phase I metabolism of this compound requires a ring hydroxylation of lignocaine.

Diagram to Show the Formation of 3-OH LIG

Lignocaine



Lignocaine 3-hydroxy Lignocaine

FIG. 3.1.3. To Show the Calculation of Metabolite Recovery.

FORM	METABOLITE CONCENTRATION nM/ml
FREE	3OHLIG + 3OHMEGX + MEGX + GX = TOTAL UNCONJUGATED METABOLITES
	+ + + +
GLUCURONIDES	3OHLIG + 3OHMEGX + MEGX + GX = TOTAL GLUCURONIDES
	+ + + +
SULPHATES	3OHLIG + 3OHMEGX + MEGX + GX = TOTAL SULPHATES
	TOTAL 3OHLIG TOTAL 3OHMEGX TOTAL MEGX TOTAL GX

TOTAL 3OHLIG + TOTAL 3OHMEGX + TOTAL MEGX + TOTAL GX

+ TOTAL OUTPUT LIGNOCAINE

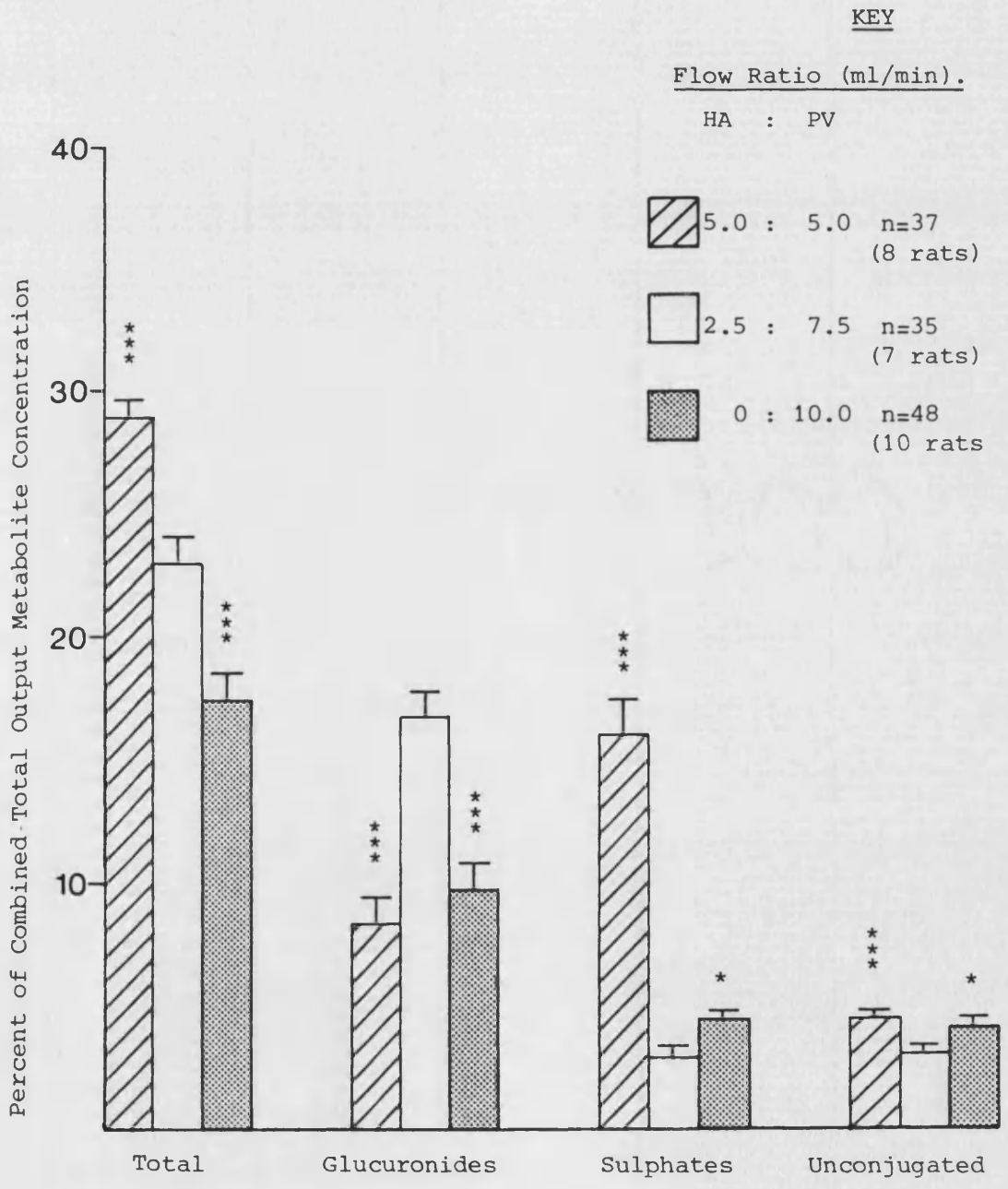
=

COMBINED TOTAL OUTPUT METABOLITE CONCENTRATION

All metabolite concentrations are expressed as a % of combined total output metabolite concentration.

Figure 3.1.4

To Show the Effect of Variations in the HA:PV Flow Ratio on the Recovery 3-OH Lig and its Conjugates. Values are Expressed as a Percentage of Combined Total Output Metabolite Concentration.



Bars are means + s.e.m.

Data is given in Appendix 4.

* $p < 0.025$

** $p < 0.01$

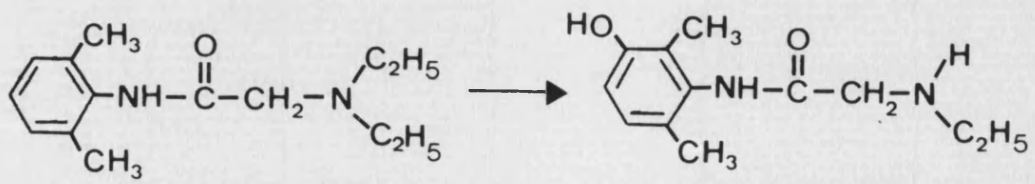
*** $p < 0.005$

Total Phase I production of 3-OH LIG shows a flow ratio dependent pattern as portal vein flow contribution is increased, so the recovery of 3-OH LIG is reduced. The pattern of glucuronide and sulphate conjugation does not follow the Phase 1 production of 3-OH LIG and so does not remain constant and although significant differences occur ($p<0.025$ and $p<0.005$) occur between the flow ratios no pattern emerges.

(ii) 3-Hydroxy Monoethylglycine Xylidide (3-OH MEGX) formation.

Figure 3.1.5. shows the recovery of 3-OH MEGX and its conjugates. The Phase I of the metabolism thus required to form this compound involves both N-deethylation and ring hydroxylation of lignocaine.

A Diagram to Show the Formation 3-OH MEGX from Lignocaine

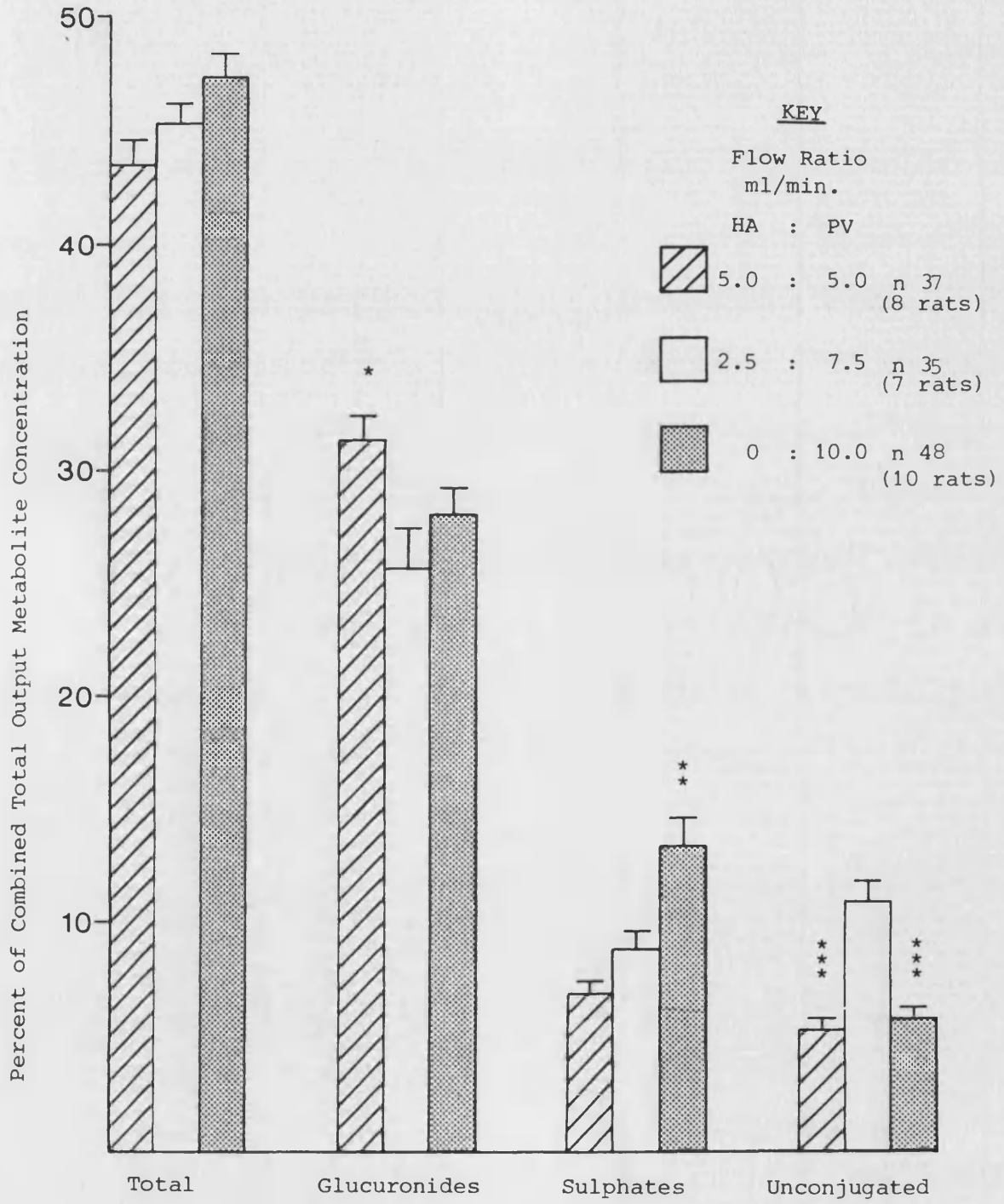


Lignocaine

3-hydroxy Monoethylglycine Xylidide

Figure 3.1.5

To Show the Effect of Variations in the HA:PV Flow Ratio on the Recovery of 3-OH MEGX and its Conjugates. Values are Expressed as a Percentage of Combined Total Output Metabolite Concentration



Bars represent the mean + sem

For data see Appendix 5

* $p < 0.025$

** $p < 0.01$

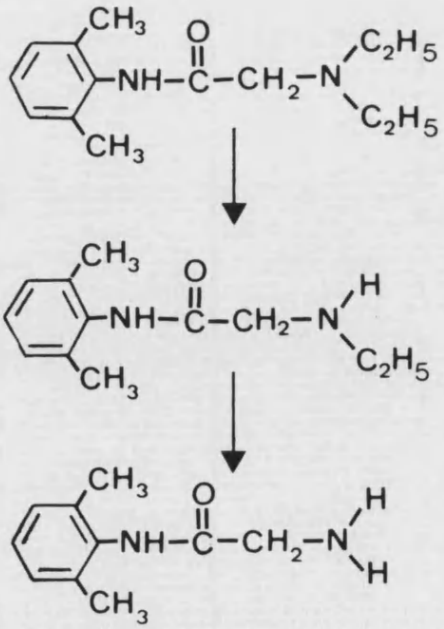
*** $p < 0.005$

The recovery of total Phase I 3-OH MEGX metabolites was not significantly flow ratio dependent. The recovery of 3-OH MEGX glucuronide also was not flow dependent. 3-OH MEGX sulphate does show an increased recovery with increasing PV flow which follows the Phase I metabolite recovery indicating constant sulphate formation. Recovery of unconjugated 3-OH MEGX was significantly higher ($p < 0.005$) at the HA:PV ratio of 2.5:7.5 ml/min.

(iii) Glycine Xylidide (GX) formation.

Figure 3.1.6 shows the recovery of GX and its conjugates. The Phase I formation of GX requires the two de-ethylation steps of lignocaine.

Diagram to Show the Formation of GX from Lignocaine



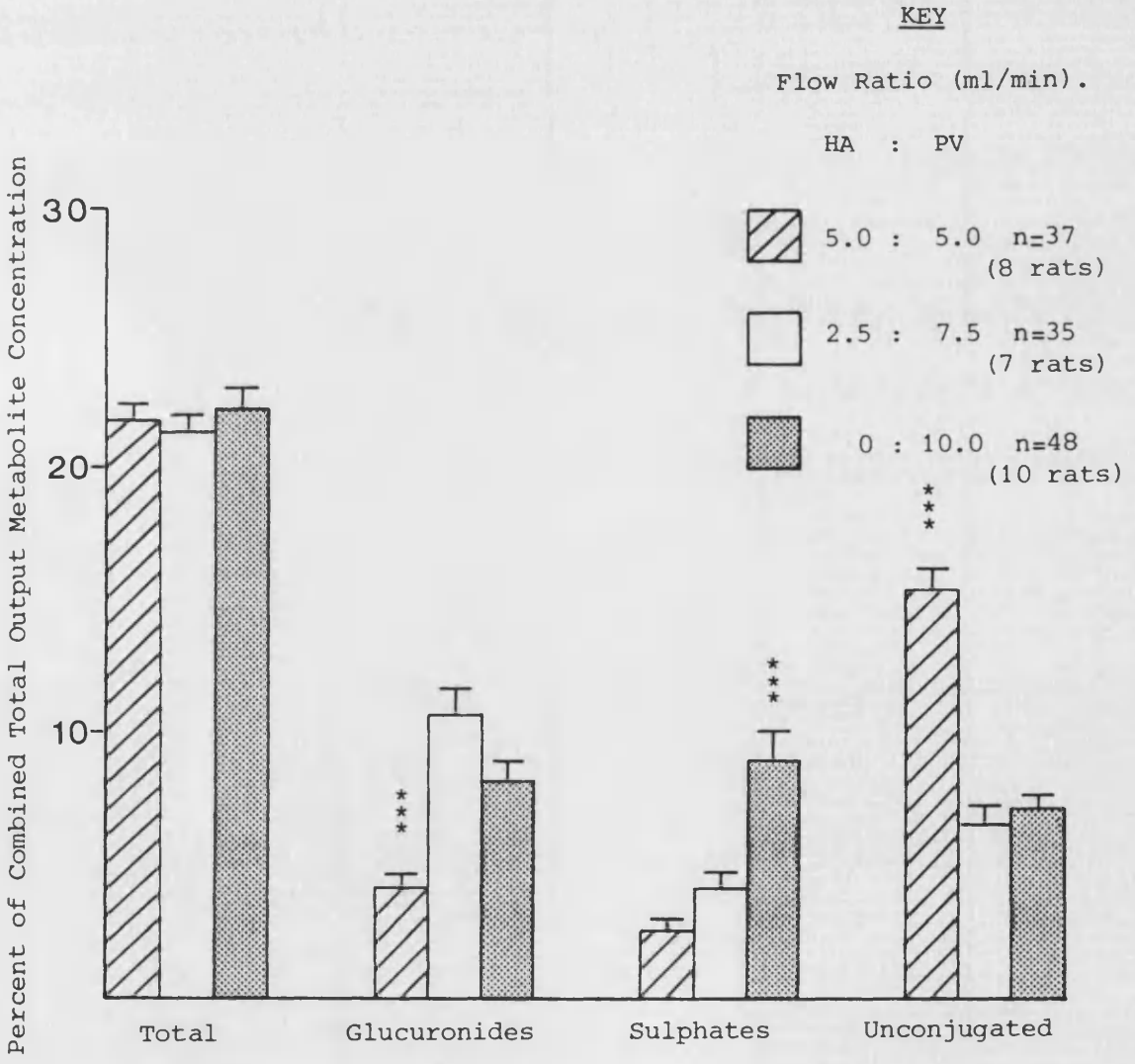
Lignocaine

Monoethylglycine Xylidide

Glycine Xylidide

Figure 3.1.6

To Show the Effect of Variations in the HA:PV Flow Ratio on the Recovery GX and its Conjugates. Values are Expressed as a Percentage of Combined Total Output Metabolite Concentration.



Bars are means + s.e.m.

Data is given in Appendix 6.

* $p < 0.025$

** $p < 0.01$

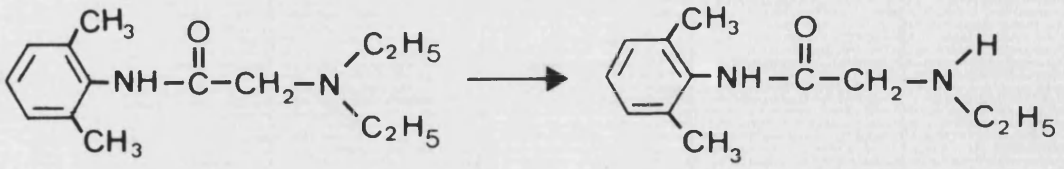
*** $p < 0.005$

Phase I production of this metabolite does not seem to be flow dependent. Recovery of GX sulphate showed some flow ratio dependency but glucuronidation did not.

(iv) Monoethylglycine Xylidide (MEGX) formation.

Figure 3.1.7 shows the recovery of the various forms of MEGX. Phase I formation requires only 1 deethylation step from lignocaine and MEGX is thought to be an intermediate for both 3-OH MEGX and GX (Keenaghan et al., 1972).

Diagram to Show the Formation of MEGX from Lignocaine

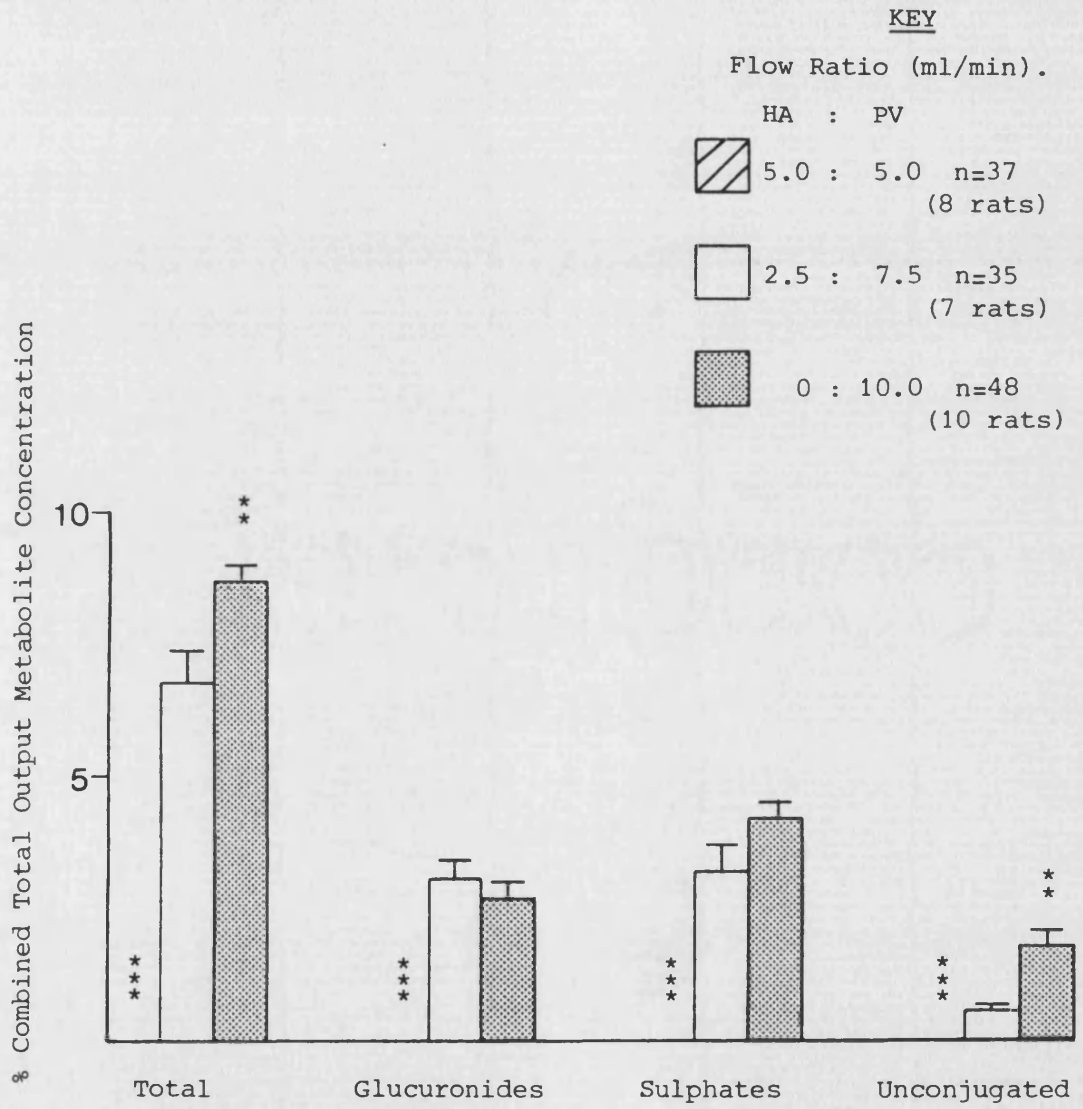


Lignocaine Monoethylglycine Xylide

MEGX was only detected in low concentrations and at the HA:PV flow ratio of 5:5 ml/min was undetectable. Total recovery of MEGX shows a flow ratio dependent increase as portal contribution is

Figure 3.1.7

To Show the Effect of Variations in the HA:PV Flow Ratio on the Recovery MEGX and its Conjugates. Values are Expressed as a Percentage of Combined Total Output Metabolite Concentration.



Bars are means + s.e.m.

Data is given in Appendix 7.

* $p < 0.025$

** $p < 0.01$

*** $p < 0.005$

raised. The recovery of free MEGX did show a flow ratio dependent pattern but both its conjugates did not.

(v) De-ethylated metabolite recovery.

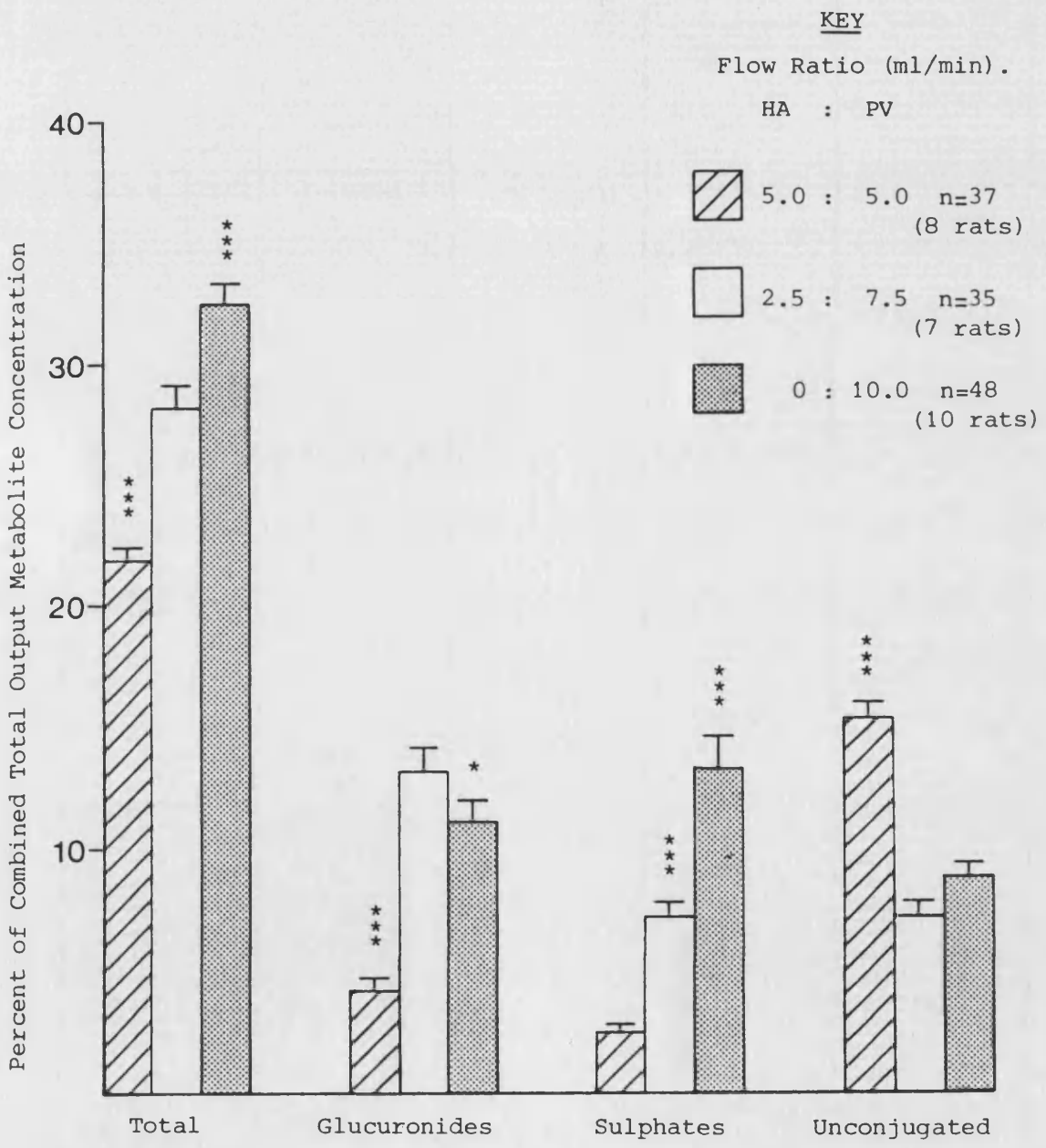
Formation of MEGX and GX require N-de-ethylation of lignocaine, the concentrations of these metabolites when combined would give an indication of any changes in de-ethylated metabolite recovery. These results are shown in Figure 3.1.8. The histogram shows that the total recovery of deethylated metabolites significantly increases ($p < 0.005$) as PV flow ratio increases. Sulphate metabolites follow a similar pattern indicating a constant sulphate production but glucuronide and unconjugated metabolite formation did not show any significant pattern.

(vi) Phase II metabolism.

Figure 3.1.9 shows the recovery of total glucuronide, sulphate and unconjugated lignocaine metabolites at the three flow ratios. No real flow dependent pattern emerges for either conjugation processes. Glucuronidation formation was shown to be significantly higher ($p < 0.005$) and sulphate formation significantly lower ($p < 0.005$) at the 2.5:7.5, HA:PV flow ratio. The recovery of unconjugated metabolites

Figure 3.2.8

To Show the Effect of Variations in the HA:PV Flow Ratio on the Recovery of De-ethylated Metabolites and Their Conjugates. Values are Expressed as a Percentage of Combined Total Output Metabolite Concentration

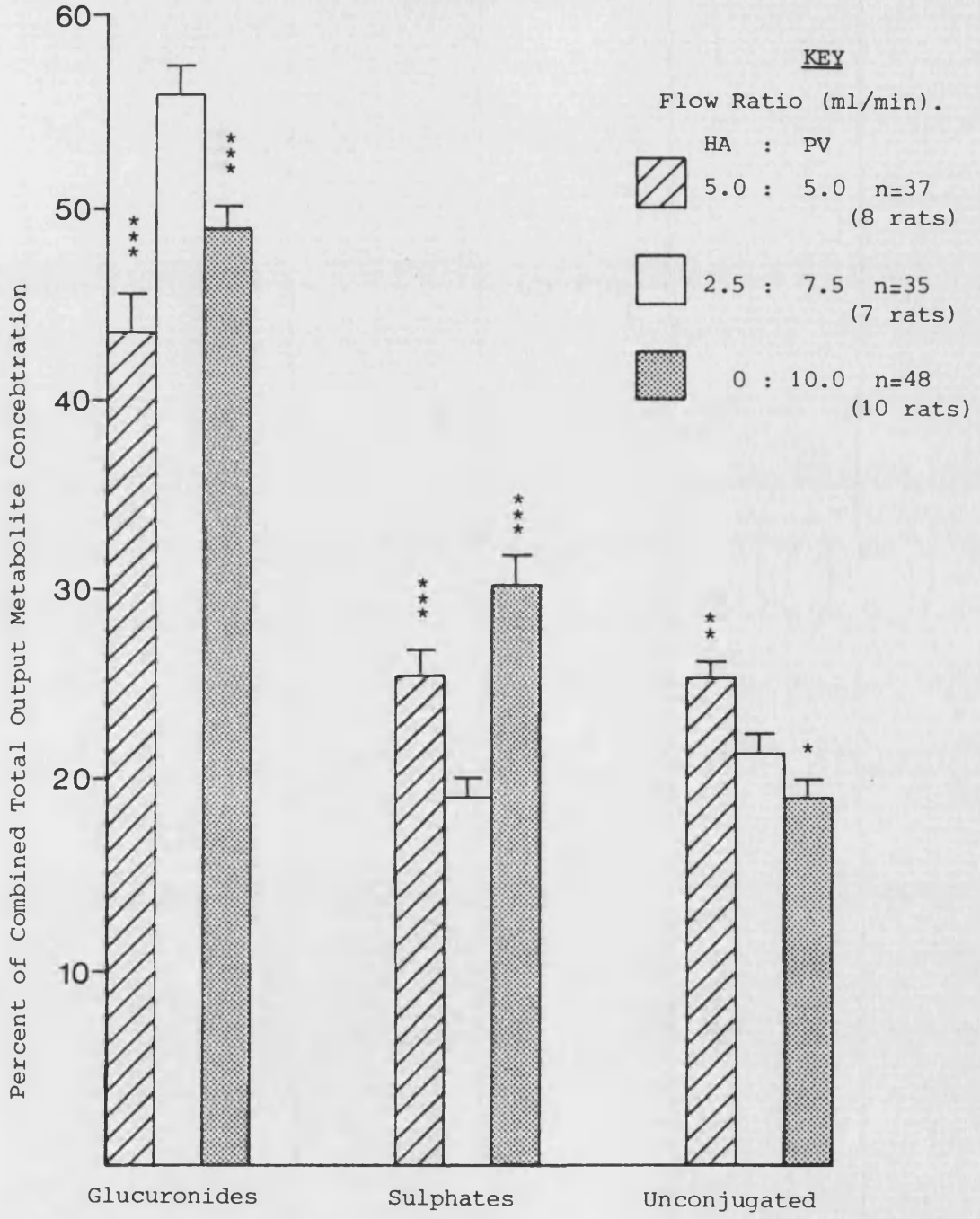


Bars represent the mean + sem.
For data see Appendix 8

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Figure 3.2.9

To Show the Effect of Variations in the HA:PV Flow Ratio on the Recovery of Conjugated and Unconjugated Metabolites. Values are Expressed as a Percentage of Combined Total Output Metabolite Concentration



Bars represent the mean + sem.
For data see Appendix 9

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

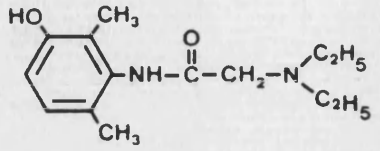
did decrease slightly as portal vein flow contribution increased.

vii) Possible explanations for the changes in lignocaine metabolite recovery during alterations in the HA:PV flow ratio.

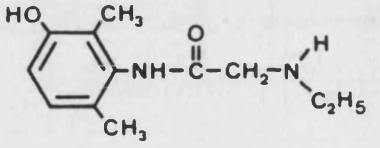
The results of figures 3.1.4 to 3.1.9 indicate that Phase I metabolite recovery shows a degree of dependency on the HA:PV flow ratio. Whilst glucuronide and sulphate conjugations do not proceed at a constant rate but during changes in flow ratio, a flow related pattern is not seen. As portal vein contribution increases the recovery of 3-OH LIG (a hydroxylated metabolite) decreases, whereas recovery of de-ethylated metabolites increases. This pattern could be the result of either an increase in de-ethylation and a decrease in hydroxylation of lignocaine as portal vein flow contribution is increased, or a decrease in further metabolism of de-ethylated metabolites and an increase in further metabolism of 3-OH LIG as portal vein flow contribution increases. 3-OH LIG is known to be further metabolised to 3-OH MEGX by a de-ethylation process (Figure 3.1.10). However, MEGX can also be further metabolised to 3-OH MEGX by a hydroxylation

Figure 3.1.10

The Conversion of 3-hydroxy-Lignocaine to 3-hydroxy-monoethylglycine Xylidide



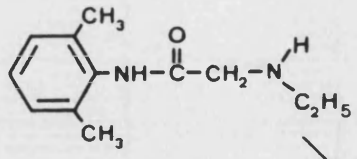
3-hydroxy Lignocaine



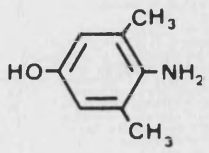
3-hydroxy monoethylglycine xylidide

Figure 3.1.11

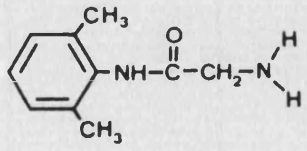
The Conversion of Monoethylglycine Xylidide to Glycine Xylidide and 2,6-Xylidine



Monoethylglycine xylidide



2,6-xylidine



Glycine xylidide

process in addition to forming GX by further de-ethylation (Figure 3.1.11). From Figure 3.1.5 it was shown that Total 3-OH MEGX recovery does not vary as portal vein flow contribution is increased. An explanation for these results might be that as portal vein flow contribution is increased the formation of MEGX from lignocaine is increased and the formation of 3-OH LIG from lignocaine is decreased. This would account for the increase in MEGX and the decrease in 3-OH LIG. If the formation of 3-OH MEGX from MEGX approximately corresponds to that from 3-OH LIG and any increase in MEGX formation from lignocaine is balanced by a decrease in 3-OH LIG formation, the net substrate concentration for 3-OH MEGX formation would remain constant. This assumes that lignocaine only produces 3-OH LIG or MEGX as its first stage Phase I metabolites and that only these metabolites can form 3-OH MEGX.

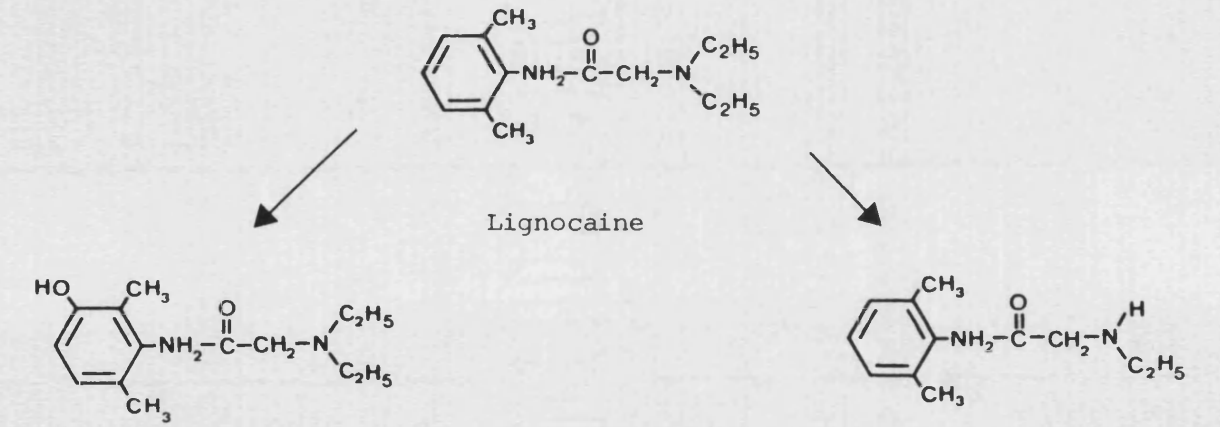
From Figure 3.1.6 it can be seen that Total GX recovery is constant as portal vein flow contribution is increased. Since it would seem that GX can only be formed by a further N-deethylation of MEGX the concentration MEGX forms the substrate for GX formation (Figure 3.1.11). From Figure 3.1.7, MEGX recovery increases. However, this does not result in an increase in GX recovery. Thus, either the rate of

formation of GX is proceeding maximally at each flow ratio and is unaffected by MEGX concentration, or any increase in MEGX concentration is balanced by a decrease in the rate of GX formation, thus keeping final GX recovery constant.

The net result of all these changes seem to be an increase in de-ethylation and a decrease in hydroxylation of lignocaine. As such, with the increase in portal vein contribution, the de-ethylation process in the assumed portal vein channels would need to be more efficient and the hydroxylation process less efficient than in the assumed hepatic artery channels.

An alternative explanation may be advanced. MEGX is known to be further metabolised in the rat (Figure 3.1.11) to metabolites such as 2,6 xylidine, not measured in this study. 3-OH LIG, 3-OH MEGX and GX may also undergo further metabolism to yet unidentified metabolites. Since only 61-68% of the lignocaine dose was recovered, the recovery of lignocaine metabolites was not complete and significant quantities of unidentified metabolite formation could account for this. Initially, lignocaine is thought to undergo an N-deethylation step to MEGX or a ring hydr - oxylation to 3-OH LIG. (Figure 3.1.12). The production

Figure 3.1.12. The Production of Monoethylglycine Xylidide and 3-hydroxy Lignocaine from Lignocaine



Lignocaine

3-hydroxy lignocaine

Monoethylglycine xylidide

of MEGX and 3-OH LIG may not differ between the two routes and the changes in recovery of these two metabolites may be due to changes in their further metabolism. These changes if route dependent may show up as a route dependency of 3-OH LIG and MEGX recovery but would not be due to a route dependency of their production. The metabolic steps responsible for further metabolism of 3-OH LIG and MEGX may be more important in determining HA:PV flow ratio dependent metabolite recovery.

In order to explain the results obtained using the hypothesis of changes in further 3-OH LIG or MEGX metabolism as portal vein flow contribution is increased, two criteria must be met. Firstly, an increase in portal vein flow contribution must result in an increase in further metabolism of 3-OH LIG thus decreasing its recovery. Secondly, an increase in portal vein contribution must result in a decrease in further MEGX metabolism resulting in an increased MEGX recovery. 3-OH LIG is known to be converted to 3-OH MEGX (Figure 3.1.10) but it is not known if it gives rise to any further Phase I metabolites. MEGX is known to produce 2,6 xylidine, 3-OH MEGX and GX (Figure 3.1.2). Since GX is recovered at a constant concentration, its formation is either proceeding at a maximum velocity

or does infact remain constant. A decrease in 3-OH MEGX production from MEGX could account for an increase in MEGX and may also cancel out any increase in 3-OH MEGX production from 3-OH LIG so that 3-OH MEGX recovery remains constant. Also, a decrease in 2,6 xylidine ation cannot be ruled out as a mechanism for increased MEGX recovery. Postulated changes in the further metabolism of hydroxylated and de-ethylated metabolites could account for the changes in lignocaine metabolite seen during alterations in the HA:PV flow ratio.

(c) Further metabolism of lignocaine metabolites.

An investigation was carried out to test the importance of interconversions between the Phase I metabolites during changes in HA:PV flow ratio. Each metabolite was infused in equimolar concentrations to 4 ug/ml lignocaine infusions at the HA:PV ratios 5:5 and 0:10 ml/min. The metabolite concentrations were expressed as a percentage of the molar dose given. Although this method of expressing the metabolite concentrations differed from that used during lignocaine infusions, GX undergoes no further metabolism and its recovery can only be expressed as a percentage of dose, so for clarity all other metabolites were

similarly expressed. Tables 3.1.2 - 3.1.5 show the recoveries of each metabolite during infusions of the various lignocaine metabolites.

(i) Changes in Phase I metabolism.

From Table 3.1.2 it can be seen that during 3-OH LIG infusion, 3-OH MEGX was the main metabolite recovered with 27-28% of 3-OH LIG undergoing no Phase I metabolism and 12-20% being recovered as unconjugated 3OHLIG. 93-95% of the input lignocaine was recovered in the output perfusate. This indicates that the metabolism of 3-OH LIG is mainly to metabolites measured in this study. 12-26% of 3-OH LIG is recovered as MEGX. The formation of MEGX from 3-OH LIG requires a reduction process (figure 3.1.13); an unusual process in drug metabolism.

Table 3.1.3 shows the metabolism of 3-OH MEGX and here the major metabolite is MEGX forming 44-68% of the recovered dose. To produce MEGX, a reduction process is required, (figure 3.1.14). 3-OH MEGX was stable in both Krebs and perfusion medium for the duration of the perfusion and the formation of MEGX from 3-OH MEGX was a product of its passage through the liver. 3-OH MEGX was also found to be stable for two months when stored at -20^{o}C in Krebs. The considerable formation of MEGX (44-68%) from 3-OH MEGX also

Table 3.1.2. The Metabolism of 3-OH Lig at the HA : PV Flow Ratios of 5:5 and 0:10

PHASE 1 METABOLITE	FLOW RATIO (ml/min) HA:PV	FORM (n=6)			
		Total	Glucuronide Conjugates	Sulphate Conjugates	Unconjugated
3-OH LIG	5 : 5	27.9 ± 1.8	4.6 ± 1.1	3.5 ± 0.7	19.8 ± 1.3
	0 : 10	26.9 ± 2.3	8.7 ± 2.0	6.3 ± 1.1	11.9 ± 1.3 **
3-OH MEGX	5 : 5	39.2 ± 2.0	11.7 ± 2.9	7.8 ± 1.1	19.6 ± 2.2
	0 : 10	56.0 ± 1.8 **	19.5 ± 2.6	13.0 ± 2.3 **	23.5 ± 1.5
MEGX	5 : 5	26.3 ± 0.7	4.8 ± 1.2	16.4 ± 1.3	5.1 ± 0.4
	0 : 10	12.1 ± 0.5 **	2.1 ± 0.8	4.9 ± 0.6 **	5.1 ± 0.4
SUM TOTAL	5 : 5	93.3 ± 1.2	21.0 ± 2.9	27.7 ± 1.1	44.5 ± 3.7
	0 : 10	95.0 ± 0.7	30.3 ± 1.5 *	33.3 ± 2.2	40.5 ± 3.0

Values are Mean % Dose of Metabolite Infused in nM ± standard error of the mean.

The significance of changes in metabolite recovery between the two flow ratios was assessed using the Wilcoxon test.

* $p < 0.025$

** $p < 0.01$

Table 3.1.3. The Metabolism of 3-OH MEGX at the HA : PV Flow Ratios of 5:5 and 0:10

PHASE 1 METABOLITE	FLOW RATIO (ml/min) HA:PV	FORM (n=6)			
		Total	Glucuronide Conjugates	Sulphate Conjugates	Unconjugated
3-OH MEGX	5 : 5	7.1 ± 0.4	1.3 ± 0.3	1.9 ± 0.3	3.9 ± 0.1
	0 : 10	16.6 ± 0.7 **	2.8 ± 0.6	1.7 ± 0.5	12.1 ± 0.4 **
MEGX	5 : 5	68.0 ± 2.6	17.7 ± 2.9	29.7 ± 0.9	20.5 ± 0.5
	0 : 10	43.8 ± 5.0 **	18.5 ± 5.7	2.5 ± 0.4 **	22.7 ± 0.4 **
GX	5 : 5	8.9 ± 0.6	0.1 ± 0.1	0.8 ± 0.5	8.0 ± 0.1
	0 : 10	8.2 ± 0.1	0.1 ± 0.1	1.4 ± 0.4	6.7 ± 0.5
SUM TOTAL	5 : 5	83.8 ± 2.6	18.9 ± 2.8	32.5 ± 1.5	32.4 ± 0.5
	0 : 10	68.6 ± 5.2 *	21.4 ± 5.7	5.6 ± 0.6 **	41.5 ± 0.8 **

Values are Mean % Dose of Metabolite Infused in nM ± standard error of the mean.

The significance of changes in metabolite recovery between the two flow ratios was assessed using the Wilcoxon test.

* $p < 0.025$

** $p < 0.01$

Table 3.1.4. The Metabolism of MEGX at the HA : PV Flow Ratios of 5:5 and 0:10

PHASE 1 METABOLITE	FLOW RATIO (ml/min) HA:PV	FORM (n=6)			
		Total	Glucuronide Conjugates	Sulphate Conjugates	Unconjugated
3-OH MEGX	5 : 5	18.0 ± 0.6	5.1 ± 0.6	10.4 ± 0.8	2.4 ± 0.4
	0 : 10	24.3 ± 2.2 *	11.9 ± 0.9 *	12.5 ± 1.8	1.9 ± 0.3
MEGX	5 : 5	31.6 ± 4.0	5.5 ± 2.0	2.5 ± 0.7	23.6 ± 2.0
	0 : 10	28.6 ± 2.2	2.5 ± 0.6	11.9 ± 1.6 **	14.6 ± 1.2 **
GX	5 : 5	25.9 ± 1.9	1.2 ± 0.7	2.1 ± 2.0	22.6 ± 1.1
	0 : 10	20.3 ± 1.7	0.2 ± 0.2	0.4 ± 0.4	19.8 ± 2.0
SUM TOTAL	5 : 5	75.5 ± 4.2	11.9 ± 1.9	15.1 ± 1.4	48.5 ± 2.5
	0 : 10	73.5 ± 2.7	14.6 ± 0.9 *	24.9 ± 0.8	36.2 ± 2.6 **

Values are Mean % Dose of Metabolite Infused in nM ± standard error of the mean.

The Significance of changes in metabolite recovery between the two flow ratios was assessed using the Wicoxon test.

* $p < 0.025$

** $p < 0.01$

Table 3.1.5. The Metabolism of GX at the HA : PV Flow Ratios of 5:5 and 0:10

PHASE 1 METABOLITE	FLOW RATIO (ml/min) HA:PV	FORM (n=6)			
		Total	Glucuronide Conjugates	Sulphate Conjugates	Unconjugated
GX	5 : 5	96.5 ± 1.0	0.7 ± 0.5	0.6 ± 0.6	95.2 ± 0.5
	0 : 10	88.4 ± 1.4 *	2.4 ± 1.0	0.9 ± 0.5	80.0 ± 1.8 **

Values are Mean % Dose of Metabolite Infused in nM ± standard error of the mean.

The significance of changes in metabolite recovery between the two flow ratios was assessed using the Wilcoxon test.

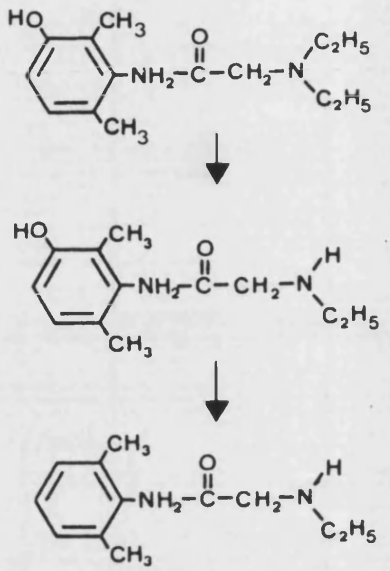
* $p < 0.025$

** $P < 0.01$

Figure 3.1.13

The Production of Monoethylglycine Xylidide

From 3-hydroxy Lignocaine



3-hydroxy lignocaine

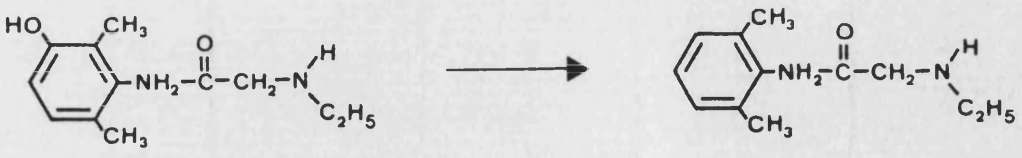
3-hydroxy monoethylglycine xylidide

Monoethylglycine xylidide

Figure 3.1.14

The Production of Monoethylglycine Xylidide

From 3-hydroxy Monoethylglycine Xylidide



3-hydroxy monoethylglycine xylidide

Monoethylglycine xylidide

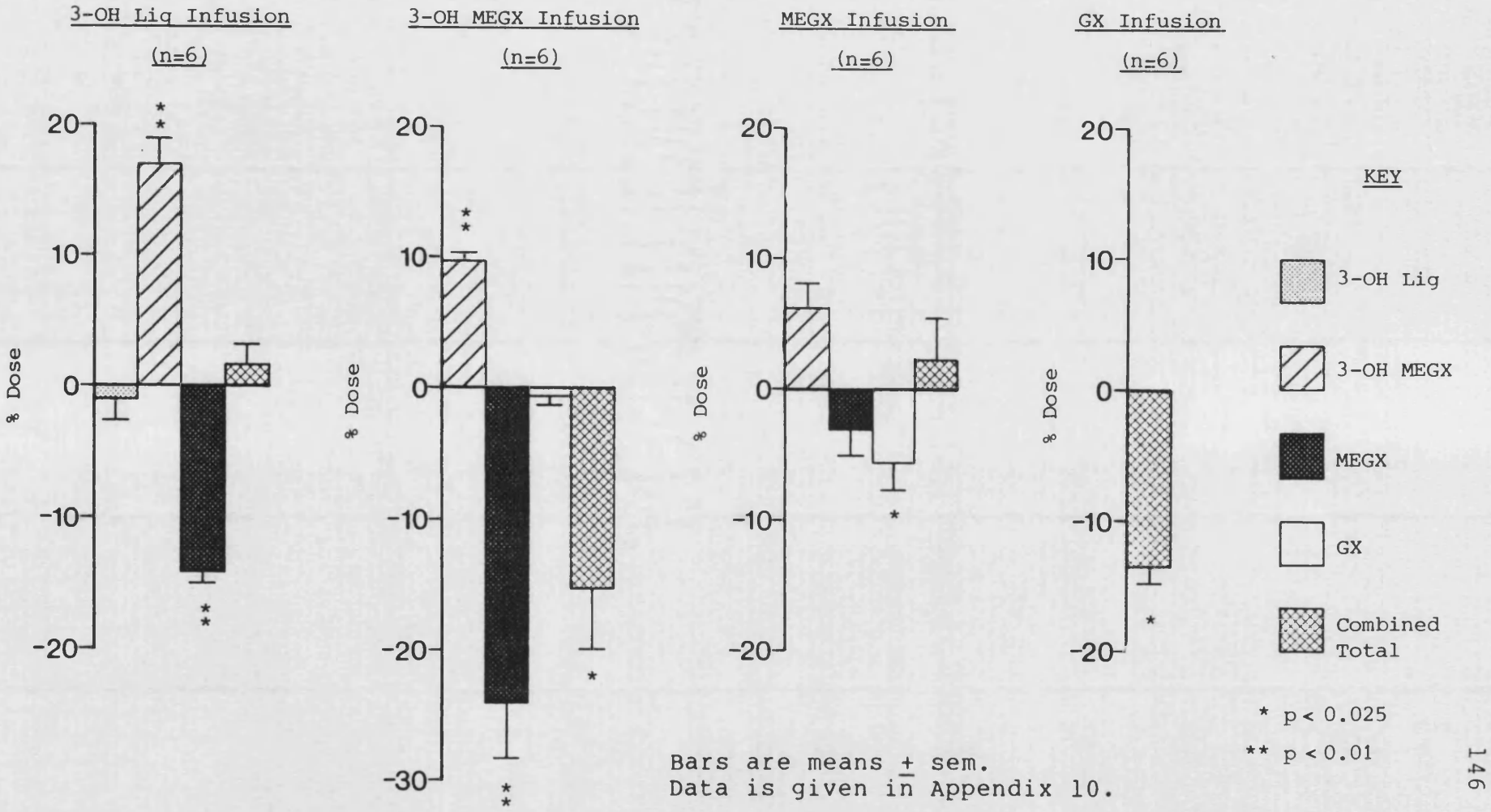
leads to the formation of GX (8.2-18.9%). 69-84% of the 3-OH MEGX dose was recovered . The lack of complete recovery may reflect further 3-OH MEGX metabolism to unidentified metabolites. 7-17% of the input dose underwent no Phase I metabolism and 4-12% of the 3OHMEGX dose was recovered unchanged.

Table 3.1.4 shows the metabolism of MEGX. MEGX forms both GX and 3-OH MEGX in approximately equal quantities. Around 30% of the infusion undergoes no Phase I metabolism and 15-24% is recovered unchanged. 74-76% of the dose was recovered and the possibility that 2,6 xylidine or other unmeasured metabolites are are formed from MEGX in significant quantities cannot be ruled out.

Table 3.1.5 shows the results of GX infusions. Further metabolism of GX does not seem to occur to any great extent as 80-95% of the drug is recovered unchanged.

In order to assess the changes in metabolite recovery, the change in metabolite concentration when portal vein flow contribution is increased was calculated. A positive change indicates that recovery increased as portal vein flow increased, and a negative value indicates a decrease in recovery. Figure 3.1.15 shows the effect of this change in flow ratio

Figure 3.1.15. The Effect of Changing the HA:PV Flow Ratio from 5:5 to 0:10 on the Recovery of Total Phase I Metabolites During Infusions of Various Lignocaine Metabolites.



on further Phase I metabolism of the four lignocaine metabolites.

During 3-OH LIG perfusion, recovery of 3-OH LIG was unaffected by flow, as was the total recovery of the 3-OH LIG dose. However, as portal vein flow contribution increased there was a marked increase in 3-OH MEGX recovery. This could be due to an increase in the de-ethylation of 3-OH LIG or a decrease in the reduction of 3-OH MEGX or a combination of both.

During 3-OH MEGX infusion the recovery of 3-OH MEGX increased as portal vein flow contribution increased. This was probably due in part to the decrease in reduction of 3-OH MEGX to MEGX. During MEGX infusions both 3-OH MEGX and GX were recovered. Changes in these metabolite recoveries are small but 3-OH MEGX is decreased with increased portal vein flow contribution. During GX perfusion GX was not significantly further metabolised.

The presence of a reduction process in the metabolism of lignocaine makes the results of the studies using infusions of lignocaine metabolites difficult to relate to the results during lignocaine infusions. Because of the interconversion between 3-OH MEGX and MEGX, hydroxylation and de-ethylation processes cannot easily be compared. Several pieces of

information can be inferred from the results. The recovery of 3-OH MEGX during infusions of 3-OH LIG is increased as the portal vein flow contribution is raised. Also during 3-OH MEGX infusion further metabolism of 3-OH MEGX is reduced with increased portal vein flow. During MEGX infusions the production of 3-OH MEGX was slightly increased. If we assume that the metabolism of 3-OH LIG and MEGX during lignocaine infusions are the major flow ratio dependent pathways, the results of the metabolite infusion experiments predict an increase in 3-OH MEGX production with increased portal vein contribution during lignocaine infusions. The predicted increase in 3-OH MEGX recovery being due to a reduced metabolism of 3-OH MEGX and a increase conversion of 3-OH LIG to 3-OH MEGX as portal vein flow contribution is raised. During lignocaine infusions 3-OH MEGX remains relatively constant as portal vein flow contribution is increased. It would therefore seem unlikely that changes in further metabolism are the major flow ratio dependent pathways.

The second possible explanation for the changes in metabolite profile with increases in portal vein flow contribution during lignocaine infusions, is that the initial hydroxylation and deethylation steps are the major flow ratio dependent

pathways. The results from the metabolite infusions predict an increase in 3-OH MEGX production during infusions of lignocaine as portal vein flow contribution is increased. This increase does not occur and changes in the production of 3-OH LIG and MEGX from lignocaine must counteract this predicted increase. From the metabolite infusion experiments the major pathway for 3-OH MEGX production would seem to be from 3-OH LIG. If during increases in portal vein flow contribution the production of 3-OH LIG from lignocaine is reduced and as a consequence MEGX production increased, although overall 3-OH MEGX substrate remains the same, the substrate available to the major pathway would be reduced. It would thus seem that increases in MEGX formation and decreases in 3-OH LIG formation from lignocaine ought to result in a fall in 3-OH MEGX recovery as portal vein flow contribution is increased. However, from the metabolite infusion experiments the reduction of 3-OH MEGX decreases and the production of 3-OH MEGX from 3-OH LIG increases as portal vein flow contribution is raised. These two factors could counteract the reduction in 3-OH LIG substrate available for 3-OH MEGX formation. The result being that if the main flow ratio dependent pathways involve the production of 3-OH LIG and MEGX

from lignocaine, the metabolite infusion experiments predict no change in 3-OH MEGX recovery if portal vein flow contribution is raised during lignocaine infusions. Thus it would seem that the most likely explanation for the changes in lignocaine metabolite profile is that hydroxylation of lignocaine to 3-OH LIG is decreased and de-ethylation to MEGX is increased when portal vein flow contribution is raised.

ii) Changes in phase II metabolism.

Figure 3.1.16 shows the recovery of the glucuronide conjugates of the four lignocaine phase I metabolites. During 3-OH LIG perfusions the glucuronide conjugates follow a similar pattern of changes as those seen by the phase I metabolite recoveries. The exception is 3-OH LIG which undergoes increased glucuronide conjugation as portal vein flow contibution is increased. During 3-OH MEGX perfusion the change in MEGX glucuronide formation is less than the change total Phase I production indicating a decreased glucuronidation. The glucuronides formed during MEGX infusions closely follow those changes seen in phase I recovery suggesting a constant glucuronide formation The recovery of GX glucuronide is very low.

Figure 3.1.16. The Effect of Changing the HA:PV Flow Ratio from 5:5 to 0:10 on the Recovery of Glucuronides During Infusions of Various Lignocaine Metabolites.

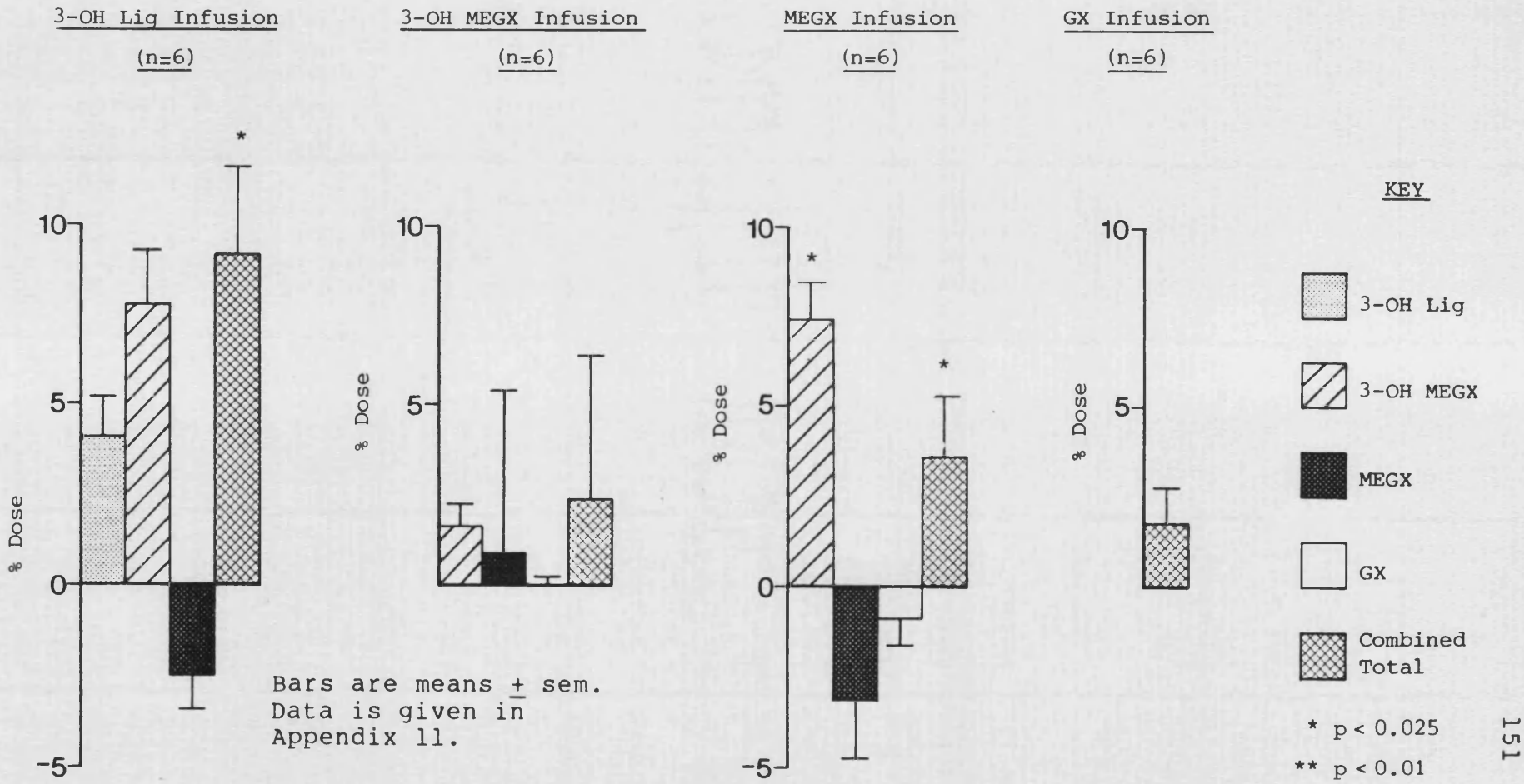


Figure 3.1.17 shows the changes in recovery of sulphate conjugates. In most cases the changes in sulphate recovery reflect the changes in phase I metabolism. Although MEGX sulphate production from 3-OH MEGX is lower at the higher portal vein flow.

iii) Changes in production of unconjugated metabolites.

Changes in the recovery of unchanged infused metabolites can be used as an assessment of the changes in extraction of the metabolites. From figure 3.1.18 the extraction of 3-OH LIG and MEGX and GX was reduced and 3-OH MEGX raised as portal vein flow contribution is increased.

Also from tables 3.1.2 - 3.1.5 it can be seen that in most cases over 40% of the metabolite dose was recovered as unconjugated metabolites. This recovery of free metabolite is higher than that seen during lignocaine perfusions; below 30% at all HA:PV flow ratios. Thus the extraction ratio of the metabolites is lower than that for lignocaine.

Figure 3.1.17. The Effect of Changing the HA:PV Flow Ratio from 5:5 to 0:10 on the Recovery of Sulphates During Infusions of Various Lignocaine Metabolites.

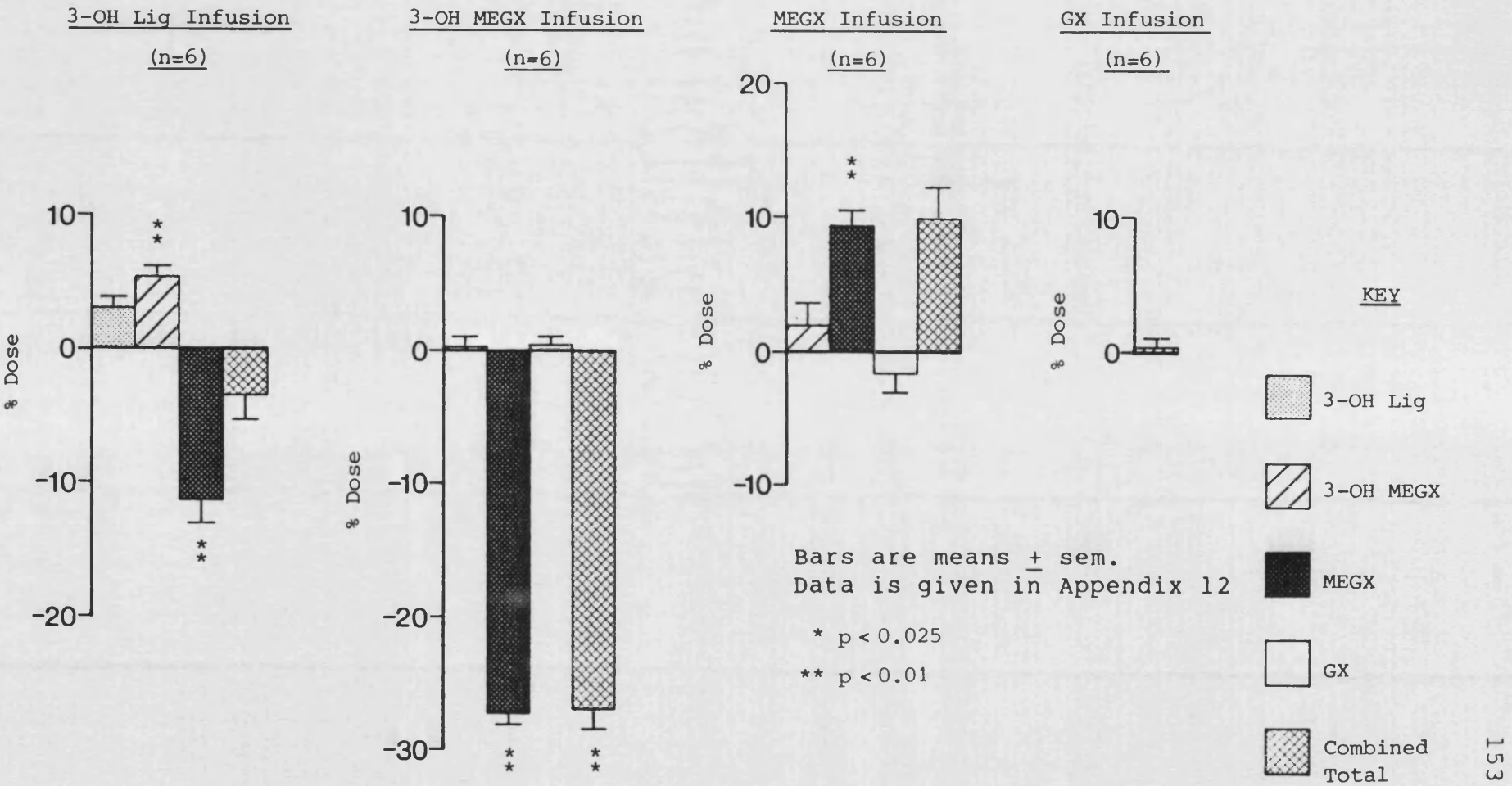
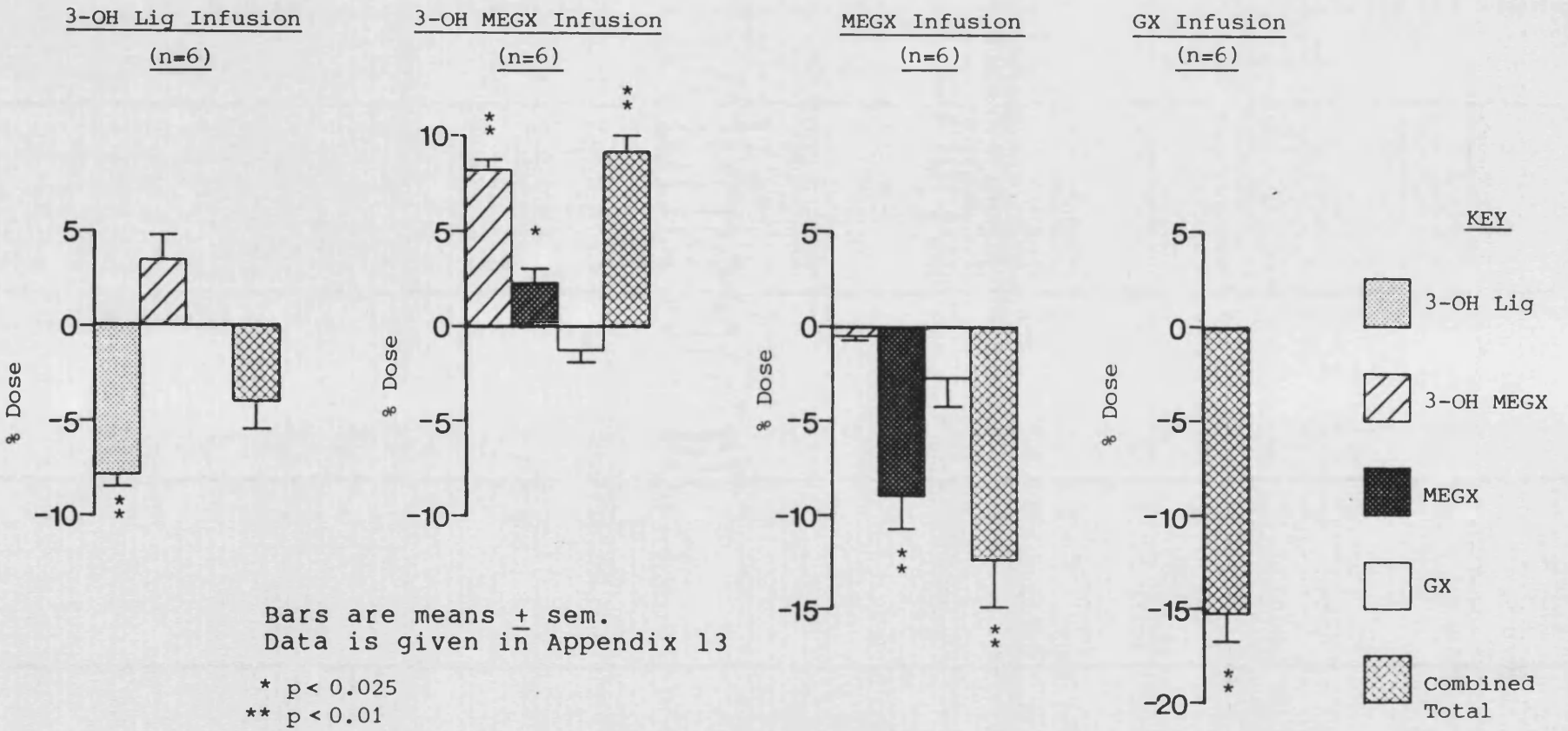


Figure 3.1.18. The Effect of Changing the HA:PV Flow Ratio from 5:5 to 0:10 on the Recovery of Unconjugated Metabolites During Infusions of Various Lignocaine Metabolites.



Bars are means ± sem.
Data is given in Appendix 13

* $p < 0.025$
** $p < 0.01$

d) Summary of the results for changes in HA:PV ratio on lignocaine metabolism.

i) Lignocaine extraction ratio increases as portal vein flow contribution is raised.

ii) The recovery of the hydroxylated metabolite 3-hydroxy lignocaine, (3-OH LIG) is decreased as portal vein flow contribution is raised.

iii) The recovery of de-ethylated metabolites increases as portal vein flow contribution is raised.

iv) The recovery of glucuronide or sulphate conjugates show no general flow ratio related pattern.

v) The major flow ratio related pathways for lignocaine seem to be the conversion of lignocaine to 3-OH LIG and MEGX.

vi) The results point to the existence of separate channels for the passages of blood through the liver and that these channels show heterogeneity in their metabolism of lignocaine.

2) DISTRIBUTION OF ADRENORECEPTORS.

(a) Reason for study.

The previous section has outlined the changes that occur in metabolism when HA:PV flow ratio is altered in the perfused rat liver. It was assumed in the discussion that the effects seen were a product of altered flow through either route. A prerequisite of an increased flow through the hepatic artery is a substantial increase in hepatic artery perfusion pressure. Portal vein pressure is independent of flow rate. Thus, changes in the metabolism of lignocaine during an increase in hepatic artery flow contribution may be caused by a raised perfusion pressure in this vessel. This increase in pressure may divert perfusate from its normal route through the liver causing drug metabolism to be altered. The changes seen in metabolism could be due to changes in in perfusion pressure rather than a reflection of increased perfusion of separate hepatic artery or portal vein channels with different metabolising environments. A model system was therefore required in the perfused rat liver where perfusion pressure could be increased or decreased at a constant flow ratio to test the effect of perfusion pressure.

One possible model was to use pharmacological vasoconstrictors and dilators to increase or decrease perfusion pressure in each vessel. Adrenoreceptor agonists have been shown to induce vasoconstriction by α stimulation in dogs (Chakravarti et al., 1940, Andrews et al., 1955, Green et al., 1959 and Noguchi et al., 1970). β-adrenoreceptor stimulation has been shown to result in dilation of the hepatic vasculature (Hirsch et al., 1976, Richardson et al., 1977d, 1978b) in dogs and by Koo et al.,(1979a) in rats.

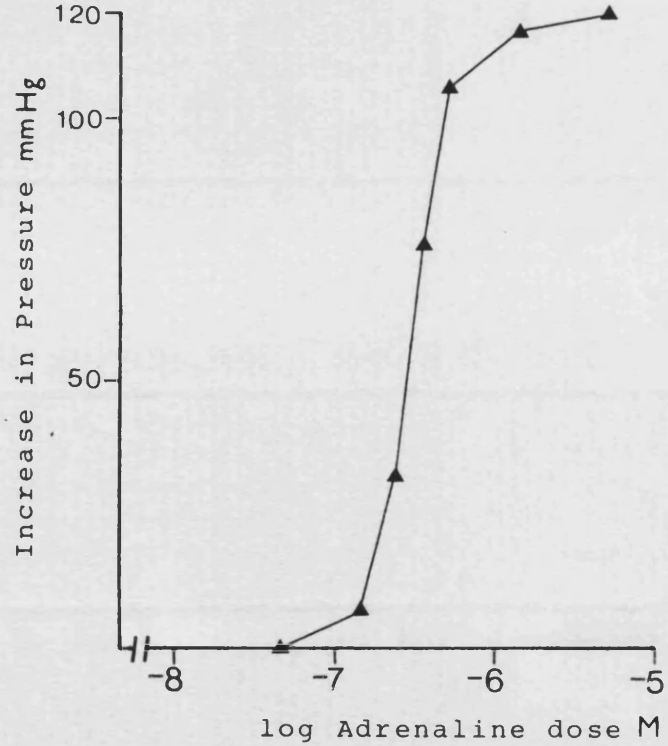
An investigation of adrenoreceptor agonism would be likely to produce a model where α-adrenoreceptor mediated vasoconstriction would produce an increase in vascular resistance increasing perfusion pressure and β -adrenoreceptor mediated vasodilation would produce a decrease in vascular resistance, decreasing perfusion pressure. An investigation of both α and β receptors was thus undertaken in both vessels using the isolated perfused rat liver at a constant HA:PV flow ratio of 2.5:7.5 ml/min which corresponds to the normal physiological flow ratio, (Greenway, 1971, and Conway et al., 1984).

(b) α-adrenoreceptor activity.

The average perfusion pressure in livers prior to any treatment was found to be 98.7 $\pm$ 3.5 mmHg in the hepatic artery and 21.5 $\pm$ 0.5 mmHg in the portal vein (values are averages of all perfusion pressures recorded with standard errors on the mean). Figure 3.2.1 shows typical log dose response curves during infusions of adrenaline in the hepatic artery or portal vein. Adrenaline gave a dose related increase in perfusion pressure in the hepatic artery and portal vein. The log dose response curves are sigmoidal in both vessels and allow the calculation of an ED50%. This represents log the dose required to produce an increase in pressure, 50% of the maximum attainable increase. Figure 3.2.2 shows typical dose response curves for noradrenaline infusions into the hepatic artery and portal vein. Noradrenaline caused a dose related increase in perfusion pressure in either vessel. The maximum increase in pressure is similar using both adrenaline and noradrenaline. Noradrenaline stimulates mainly α-adrenoreceptors, but adrenaline stimulates both α and β-adrenoreceptors. The vasoconstriction response (increase in perfusion pressure) would most likely be α mediated as the

Figure 3.2.1. Typical log Dose Response Curves for Adrenaline.

Hepatic Artery



Portal Vein

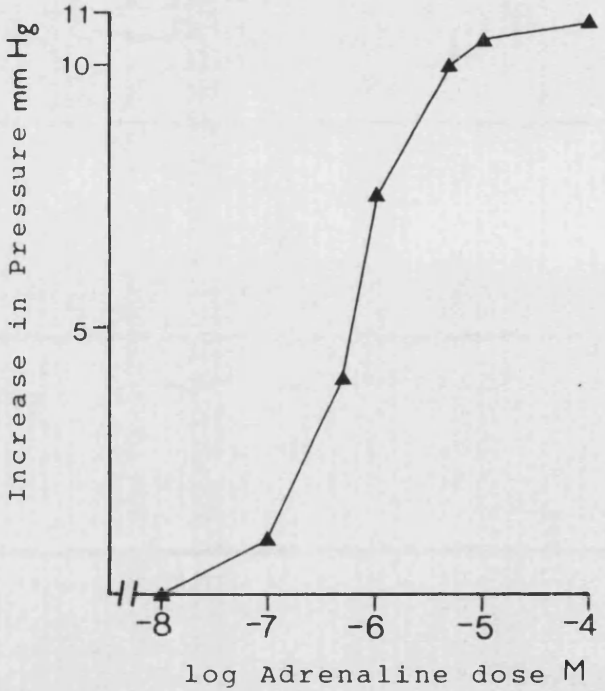
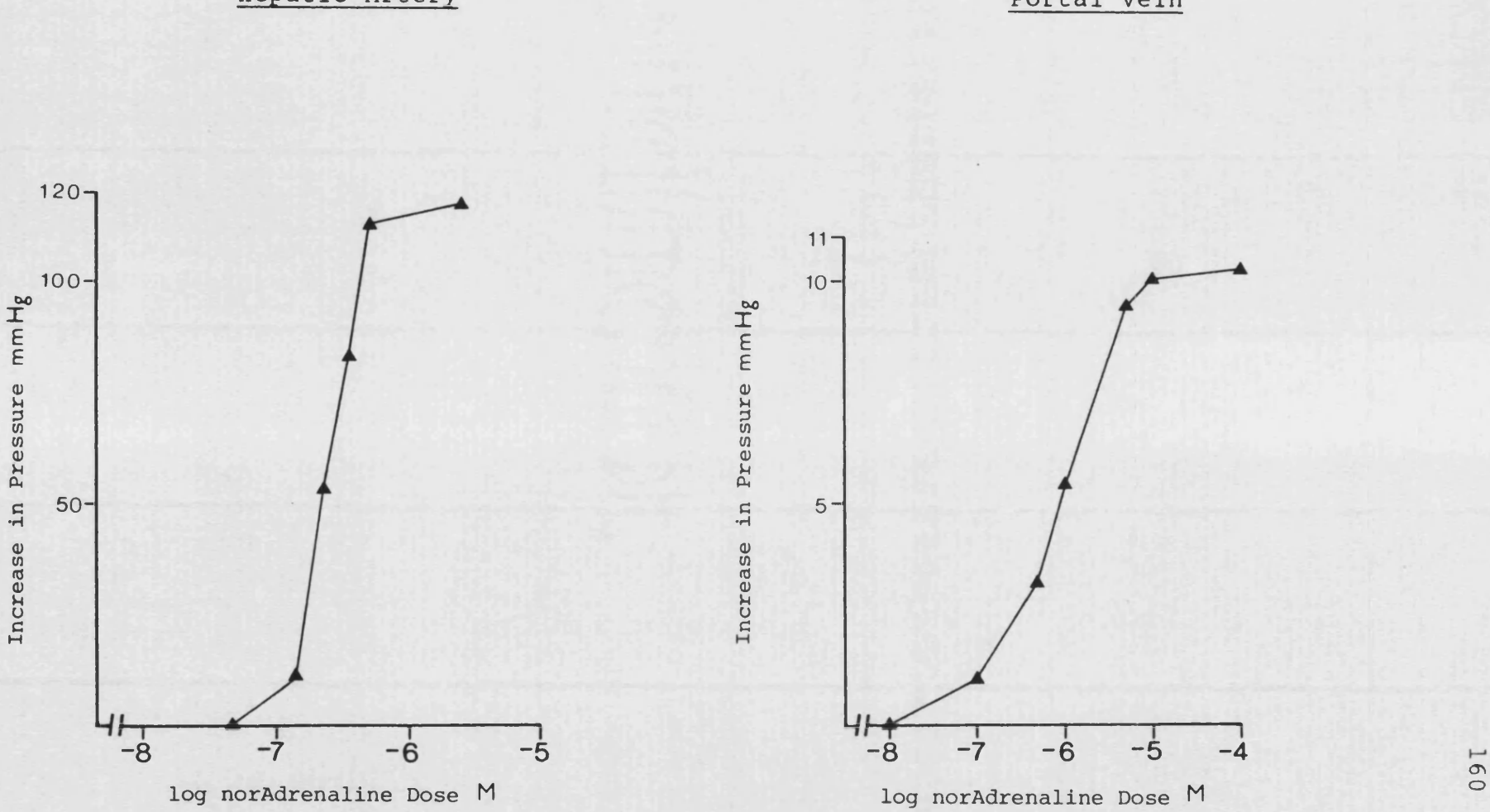


Figure 3.2.2. Typical log Dose Response Curves for norAdrenaline.

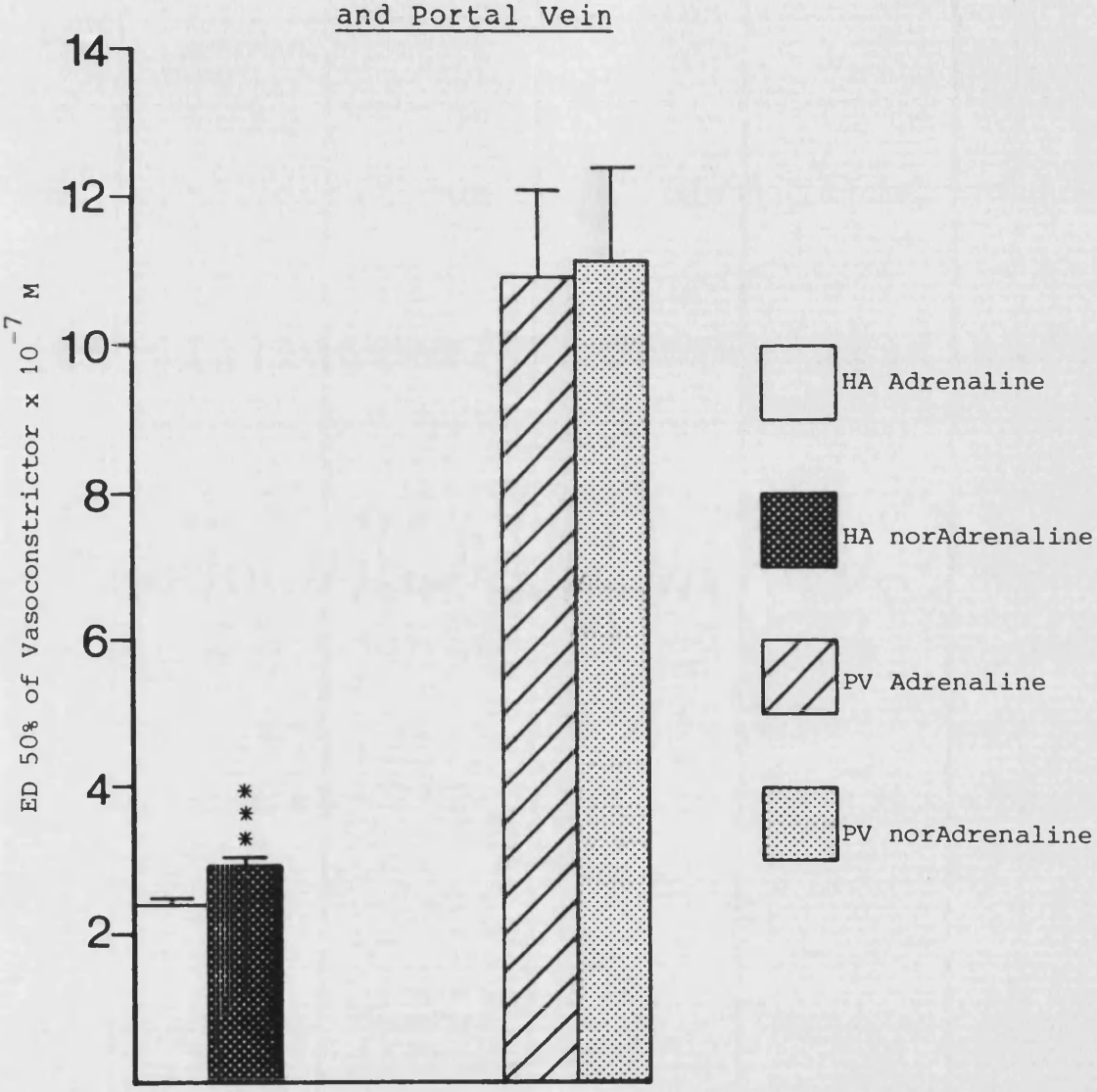


response is seen with noradrenaline as well as adrenaline. This agrees with the results from other workers, (Richardson et al., 1977a, 1978b, Koo et al., 1977 and Greenway, 1979), who showed that the hepatic vasoconstriction was α-adrenoreceptor mediated.

Figure 3.2.3 shows the average ED50% of adrenaline and noradrenaline in both vessels. The ED50% values of both adrenaline and noradrenaline are significantly lower in the hepatic artery than the portal vein. This suggests that the hepatic artery is more sensitive to adrenaline and noradrenaline vasoconstriction. Also, in the hepatic artery adrenaline has a significantly lower ED50% than noradrenaline, indicating the vessel is more sensitive to adrenaline vasoconstriction. This is unusual because noradrenaline would be expected to be more potent as constriction is α-adrenoreceptor mediated and noradrenaline is more selective. Also adrenaline stimulates β-adrenoreceptors which are thought to be dilatory and therefore would reduce the effectiveness of adrenaline on α-adrenoreceptor mediated vasoconstriction. The results are not unique. Richardson et al.,(1982) showed in the dog that the dose of noradrenaline needed to double vascular resistance was slightly less in the hepatic artery than that of adrenaline. However, in the portal

Figure 3.2.3

The ED50% Values for Adrenaline and norAdrenaline Induced Vasoconstriction in the Hepatic Artery and Portal Vein



Bars are means + sem.

*** $p < 0{\cdot}005$

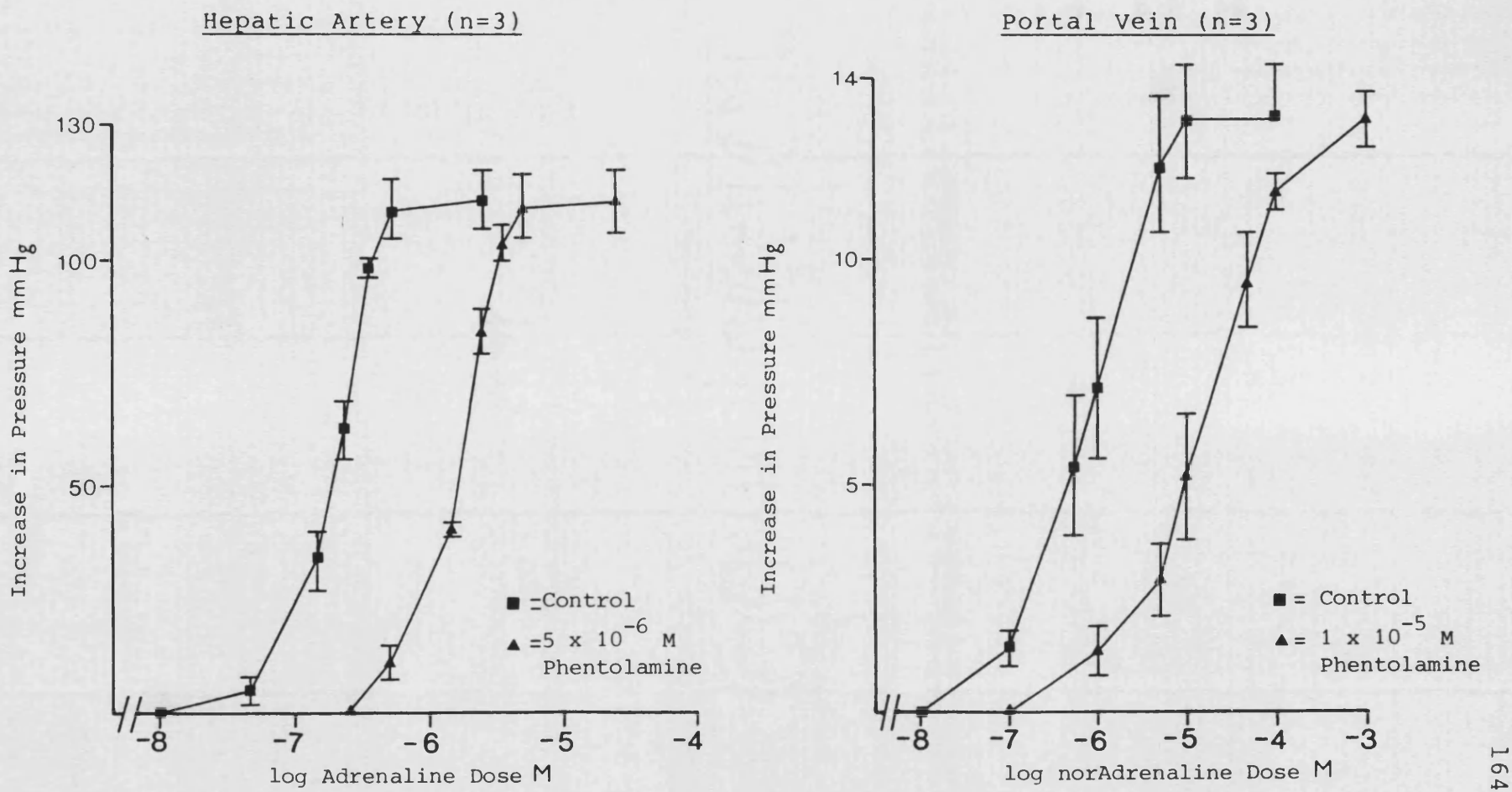
n=40 for HA

n=35 for PV

vein, a much larger dose of noradrenaline to adrenaline was required to produce a similar increase in resistance. The results may reflect different isomers of the agonists being used and their reactivity with receptors in each supply. In the present study, noradrenaline and adrenaline were equipotent in the portal vein but adrenaline was more potent in the hepatic artery.

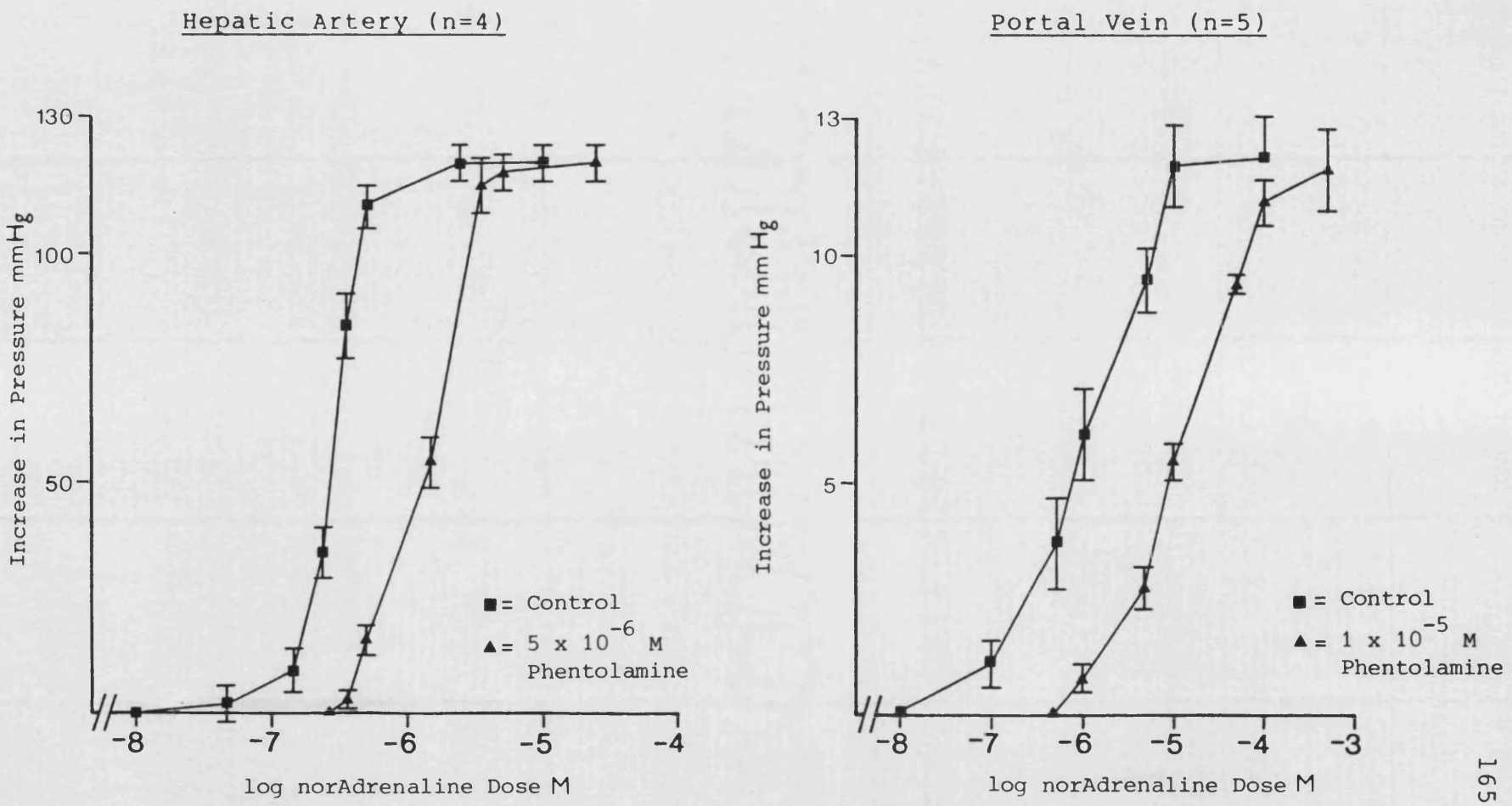
Figure 3.2.4 shows the effect of phentolamine on the log dose response curves of adrenaline. In both vessels phentolamine infusion results in parallel shift in adrenaline dose response curve. Figure 3.2.5 shows the effect of phentolamine on the noradrenaline dose response curve. The results show a similar parallel shift to the right. These results indicate that phentolamine competitively antagonises the vasoconstriction induced by both constrictors. Since phentolamine is a specific α-adrenoreceptor antagonist, the vasoconstriction produced by adrenaline must be due to stimulation of α-adrenoreceptors Richardson et al.,(1977a, 1978b) in dogs and Greenway (1979) in cats showed a similar phentolamine antagonism of constriction in both the hepatic artery and portal vein.

Figure 3.2.4. The Effect of Phentolamine on the log Dose Response Curves of Adrenaline in the Liver Vasculature.



points are means ± sem.

Figure 3.2.5. The Effect of Phentolamine on the log Dose Response Curves of norAdrenaline in the Liver Vasculature.



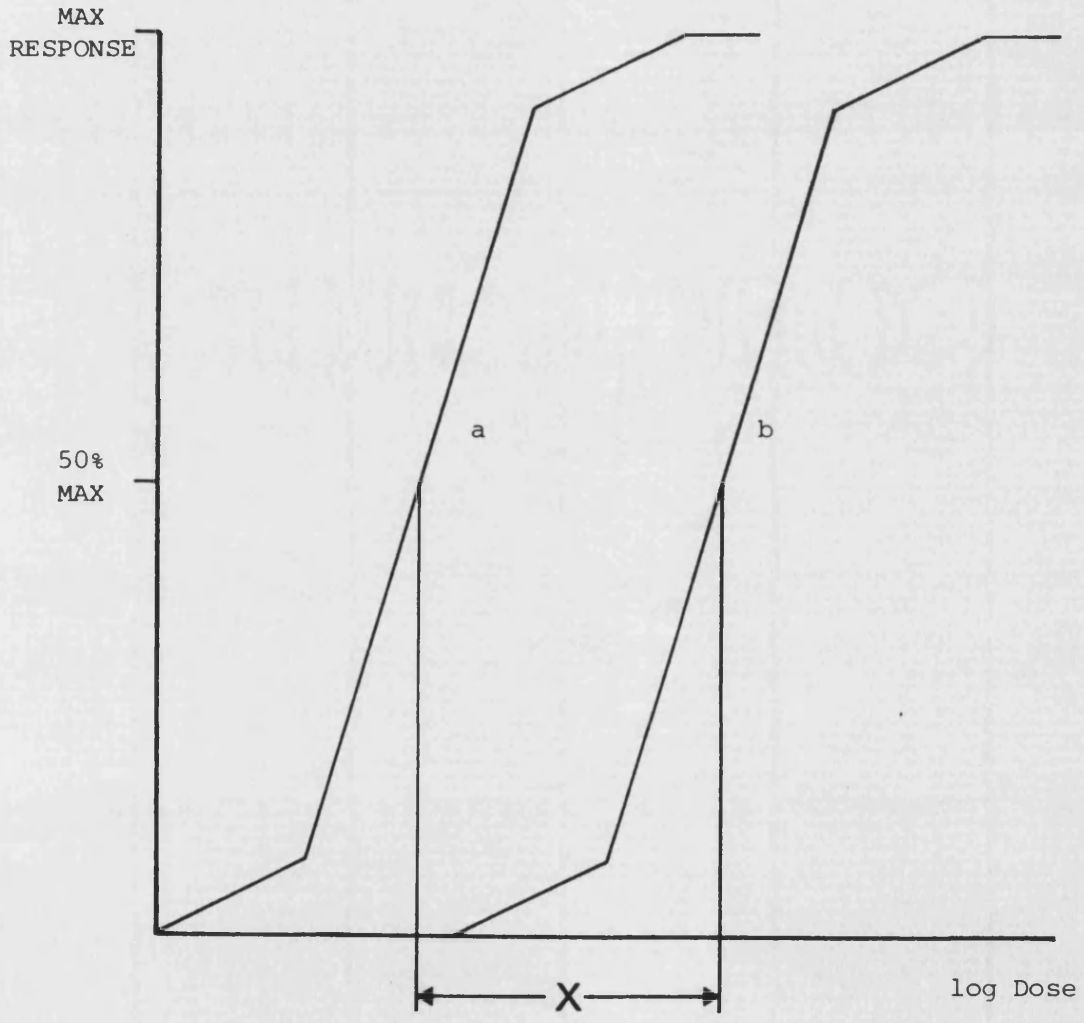
Points are means ± sem.

As the dose response curves show a parallel shift, an ED50% can be calculated for both vasoconstrictors in the presence of phentolamine. The degree of antagonism due to phentolamine can be represented by the shift in ED50% from the unantagonised to antagonised log dose response curves. The calculation of this change is represented schematically by Figure 3.2.6. Curve "a" represents the normal unantagonised dose response curve for adrenaline or noradrenaline. Curve "b" represents the curve during infusions of phentolamine. The difference in the ED50% of the two curves is represented by "X". The value of "X" can be used as a measure of the degree of antagonism. The larger the shift in ED50% the higher the value of "X" and the greater the antagonism.

The results of the effect of various concentrations of phentolamine on the log dose response curves of adrenaline and noradrenaline are given in figure 3.2.7. A dose related antagonism by phentolamine on both constrictors is seen in both the hepatic artery and the portal vein. The antagonism of noradrenaline induced hepatic artery constriction due to 5×10^{-6} M infusions of phentolamine is larger then that caused by 1×10^{-5} M infusions of phentolamine

Figure 3.2.6

A Schematic Representation to Show the Calculation of the Shift in ED50% of Constrictor Due to Infusions of Phentolamine

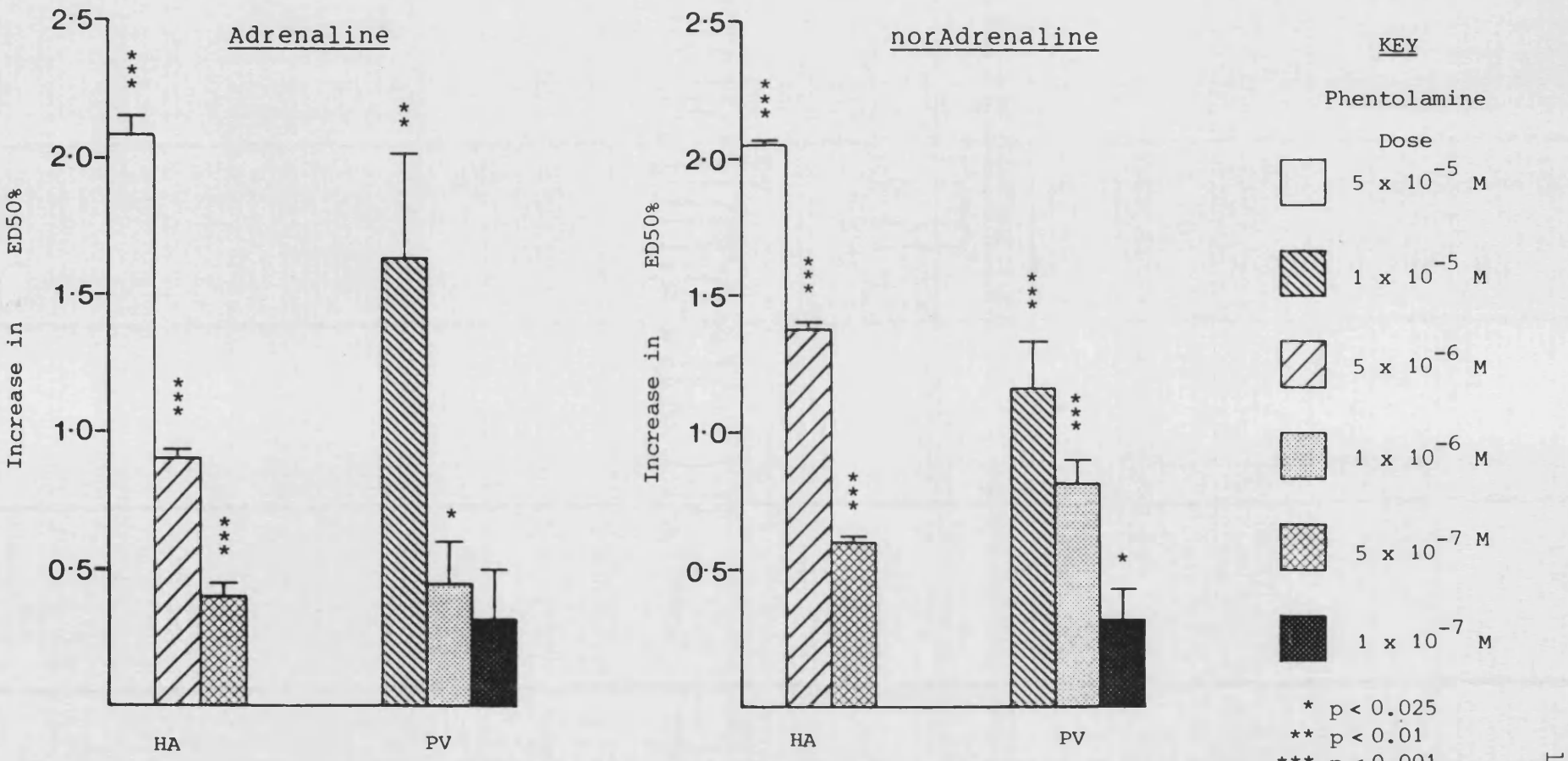


"a" = the normal constrictor log dose response curve.

"b" = the constictor log dose response curve in the presence of Phentolamine.

"X" = the difference in ED50% of curves "a" and "b".

Figure 3.2.7. The Effect of Hepatic Artery and Portal Vein Infusions of Phentolamine on the ED50% of Adrenaline and norAdrenaline



Bars are means + sem. Data is given in Appendixes 14 and 15

in the portal vein. This suggests that phentolamine may be less effective at antagonising noradrenaline vasoconstriction in the portal vein.

Tables 3.2.1 and 3.2.2 show the effect of infusions of β-adrenoreceptor blockers on the ED50% of adrenaline and noradrenaline in the hepatic artery and the portal vein. Several β-adrenoreceptor blocking drugs were infused. All show a degree of β_1 and β_2 activity but atenolol, metoprolol and oxprenolol are relatively β_1 selective. Propranolol shows a greater mixed activity but is more β_1 selective and ICI 118 551 is a selective β_2-adrenoreceptor blocking drug, (Arnold et al., 1985; Bilski et al., 1983). Propranolol was the only β blocker tested which had a significant effect ($p < 0.005$), on the dose response curves of adrenaline in the hepatic artery. This may be due to partial β-adrenoreceptor agonist activity, although at the concentration used (5×10^{-5} M), these effects would be small. All β blockers had no significant effect on the log dose response curves of noradrenaline in the hepatic artery (table 3.2.2). Portal vein infusions of the β blockers produced no significant effects on the log dose response curves of adrenaline or noradrenaline (table 3.2.3).

Table 3.2.1. The Effect of Beta Adrenoreceptor blockers on the ED50% of Adrenaline induced Vasoconstriction in the Hepatic Artery.

	CHANGE IN ED50% FROM CONTROL		
	Infusion Concentration		
BLOCKER	5×10^{-5} M n=3	5×10^{-6} M n=3	5×10^{-7} M n=3
Atenolol	0.04 ± 0.03	0.01 ± 0.01	-
Propranolol	0.26 ± 0.03 ***	-0.05 ± 0.07	-0.05 ± 0.05
Metoprolol	0.01 ± 0.01	0.01 ± 0.02	-
Oxprenolol	0.15 ± 0.08	0.07 ± 0.06	0.01 ± 0.01
ICI 118 551	0.16 ± 0.06	0.01 ± 0.01	0.01 ± 0.02

Values are mean changes in ED50% ± standard error of the mean.

Significance was assessed using the related "t" test

*** $p < 0.005$

Table 3.2.2. The Effect of Beta Adrenoreceptor blockers on the ED50% of norAdrenaline induced Vasoconstriction in the Hepatic Artery.

	CHANGE IN ED50% FROM CONTROL	
	Infusion	Concentration
BLOCKER	5×10^{-5} M n=3	5×10^{-6} M n=3
Atenolol	0.01 ± 0.10	-0.04 ± 0.02
Propranolol	0.03 ± 0.02	-0.11 ± 0.05
Metoprolol	0.09 ± 0.02	-0.02 ± 0.02
Oxprenolol	-	-0.04 ± 0.03
ICI 118 551	0.12 ± 0.06	-0.01 ± 0.02

Values are mean changes in ED50% ± standard error of the mean.

All changes were not stastistically significant when assessed using the related "t" test.

Table 3.2.3. The Effect of Beta Adrenoreceptor blockers on the ED50% of Adrenaline and norAdrenaline induced Vasoconstriction in the Portal Vein.

	1×10^{-5} M Infusions of Beta Blockers	
	CHANGE IN ED50% FROM CONTROL	
BLOCKER	Adrenaline n=3	norAdrenaline n=3
Atenolol	0.03 ± 0.03	0.23 ± 0.11
Propranolol	0.05 ± 0.05	-0.18 ± 0.18
Metoprolol	0.07 ± 0.04	0.06 ± 0.05
Oxprenolol	0.08 ± 0.03	0.04 ± 0.03
ICI 118 551	0.02 ± 0.03	-0.01 ± 0.03

Values represent mean changes in ED50% ± standard error of the mean.

All values were not statistically significant when assessed using the related "t" test.

The lack of β-adrenoreceptor blocking drug antagonism of the log dose response curves of both vasoconstrictor agents in the hepatic artery and the portal vein indicate that the constriction induced is not β-adrenoreceptor mediated. Richardson et al. (1977a, 1978b) and Hirsch et al. (1976) in dogs found propranolol to be ineffective at antagonising adrenaline and noradrenaline induced vasoconstriction in the hepatic artery and the portal vein. The results obtained in the perfused rat liver preparation used in the present study confirm the results obtained in dogs (Richardson et al., 1977a, 1978b and Hirsch et al. 1976).

The antagonism of the adrenaline and noradrenaline log dose response curves by phentolamine together with the lack of antagonism with β blockers in the hepatic artery and the portal vein suggest that vasoconstriction is mediated by α-adrenoreceptors in both vessels.

c) β-adrenoreceptor activity.

Richardson et al. (1978b) in dogs and Reilly et al. (1981) in rats showed that β-adrenoreceptor agonists such as isoprenaline dilate the hepatic artery and the portal vein. It was found

using the perfused rat liver preparation in the present study, that infusions of β-adrenoreceptor agonists isoprenaline, salbutamol, clenbuterol and dobutamine were unable to produce a decrease in perfusion pressure. Other reported vasodilators such as histamine, (Richardson et al., 1978b) and acetylcholine, (Andrews et al., 1955), were found to be ineffective at dilating the preparation. The most likely explanation for the results obtained is that the preparation lacked any intrinsic tone and was in a state of maximal dilation. There exist several methods to induce tone in isolated preparations.

i) Catacholamines may be infused by adding them to the perfusion medium. As shown in section (b) adrenaline and noradrenaline infusions result in vasoconstriction in both the hepatic artery and the portal vein. Constant rate infusions of these agents could be used to produce a constant tone in each vessel. In order to show significant vasodilation approximately 70% maximal constriction needed to be maintained. Infusions of adrenaline and noradrenaline were found to induce a constant tone in the portal vein but a constant tone could not be maintained under such conditions in the hepatic artery. Constant infusions of vasoconstrictors in the hepatic artery were only

able to maintain a constant tone (indicated by a constant increased perfusion pressure), for approximately 10 minutes. A decrease in vasoconstriction at constant constrictor infusion was commonly seen. A similar phenomenon was reported in cats during constant hepatic nerve stimulation by Greenway et al. (1972). In order to carry out successful experiments a constant tone in the hepatic artery needed to be maintained for over an hour. This method of tone induction was unsuitable for the isolated perfused rat liver.

ii) Sympathetic nerve stimulation has also been shown to induce vasoconstriction in the hepatic vasculature, (Burton-Opitz, 1911b; Green et al., 1959; Lautt, 1977d, 1980b and Greenway et al., 1972). Tone could therefore be induced by nerve stimulation. The hepatic nerves which produce vasoconstriction when stimulated are extremely difficult to identify in the rat. Also since Greenway et al. (1972) noted vasoconstriction escape during constant nerve stimulation and as outlined in section (i) constant infusions of noradrenaline were not able to induce a constant tone, similar instability of tone might be expected during nerve stimulation.

iii) A third possible method to induce tone in isolated preparations involves changing the ionic concentrations of the perfusion medium. An increase in potassium will partially depolarise muscle cells and induce smooth muscle tone. Potassium chloride infusions between 0.1M and 0.2M were required to induce sufficient tone. Using these concentrations of potassium chloride an increase in haemolysis of red blood cells during perfusion was seen. This reduced the viability of the preparations and could not be used as a method to induce tone.

iv) An indirect method for measuring β-adrenoreceptor activity was found to be effective. It was noted that β-adrenoreceptor agonists when infused during construction of adrenaline or noradrenaline log dose response curves shifted the curves to the right, (figures 3.2.8 and 3.2.9). This shift was due to β-adrenoreceptor mediated vasodilation physiologically antagonising the α-adrenoreceptor mediated vasoconstriction. The parallel shift in log dose response curve for both constrictors may be compared using the shift in ED50% as shown in figure 3.2.10. The shift in ED50% in figure 3.2.10 is represented by "X", the larger the value the greater the β-adrenoreceptor mediated activity.

Figure 3.2.8. The Effect of Isoprenaline on the log Dose Response Curves of Adrenaline in the Liver Vasculature.

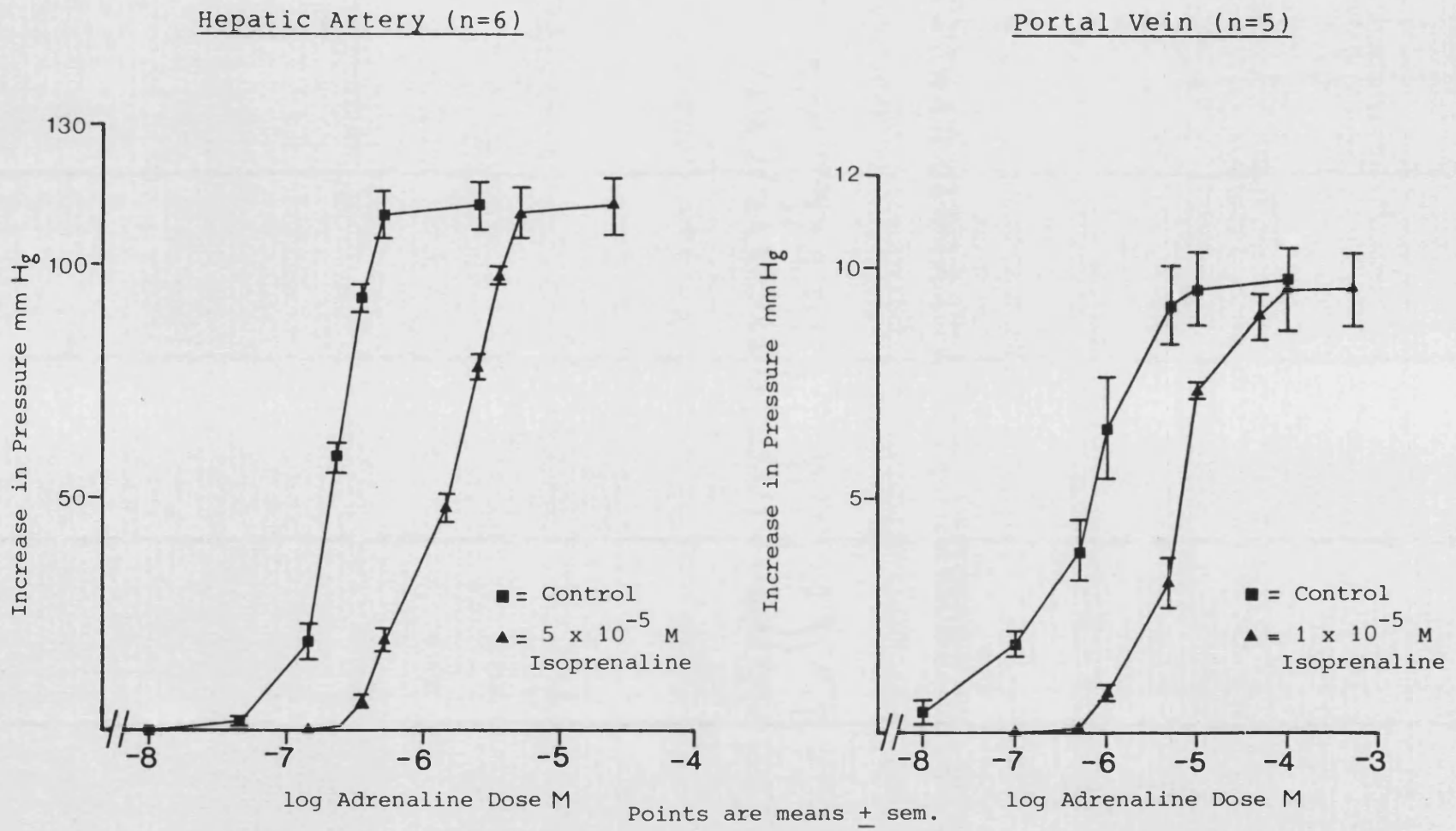
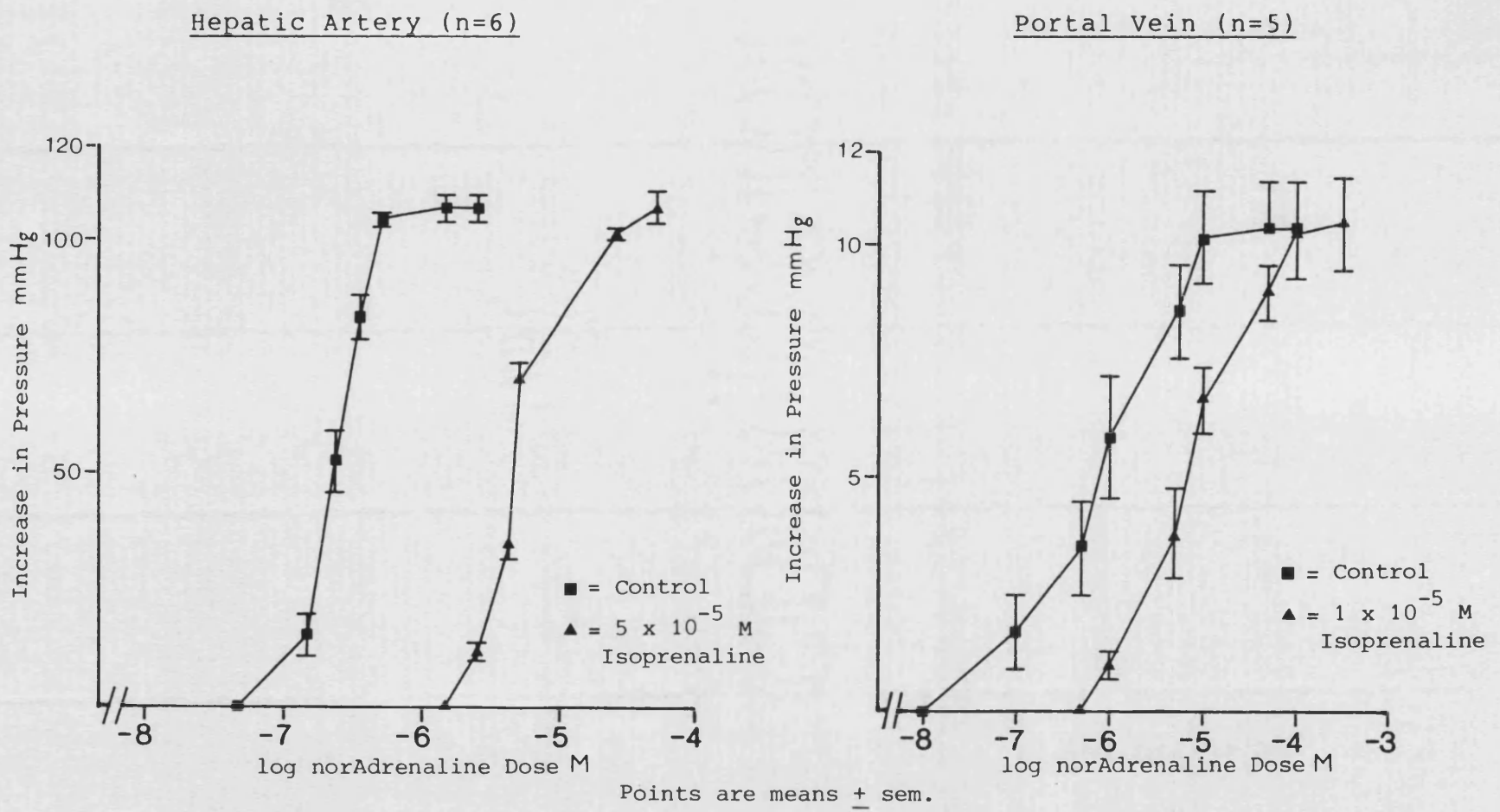


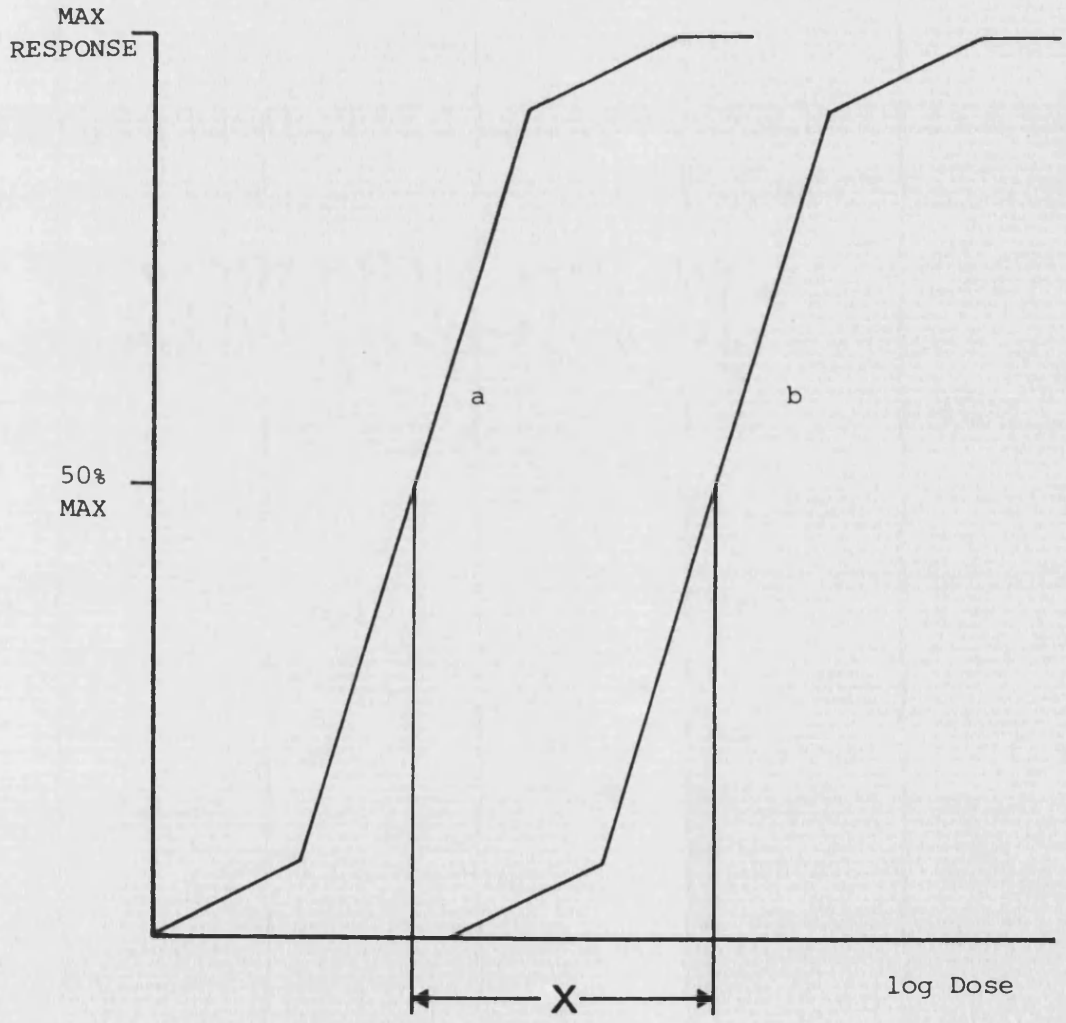
Figure 3.2.9. The Effect of Isoprenaline on the log Dose Response Curves of norAdrenaline in the Liver Vasculature.



Points are means ± sem.

Figure 3.2.10

A Schematic Representation to Show the Calculation of the Shift in ED50% of Constrictor Due to Infusions of β-Adrenoreceptor Agonists.



"a" = the normal constrictor log dose response curve.

"b" = the constictor log dose response curve in the presence of β-Adrenoreceptor Agonists.

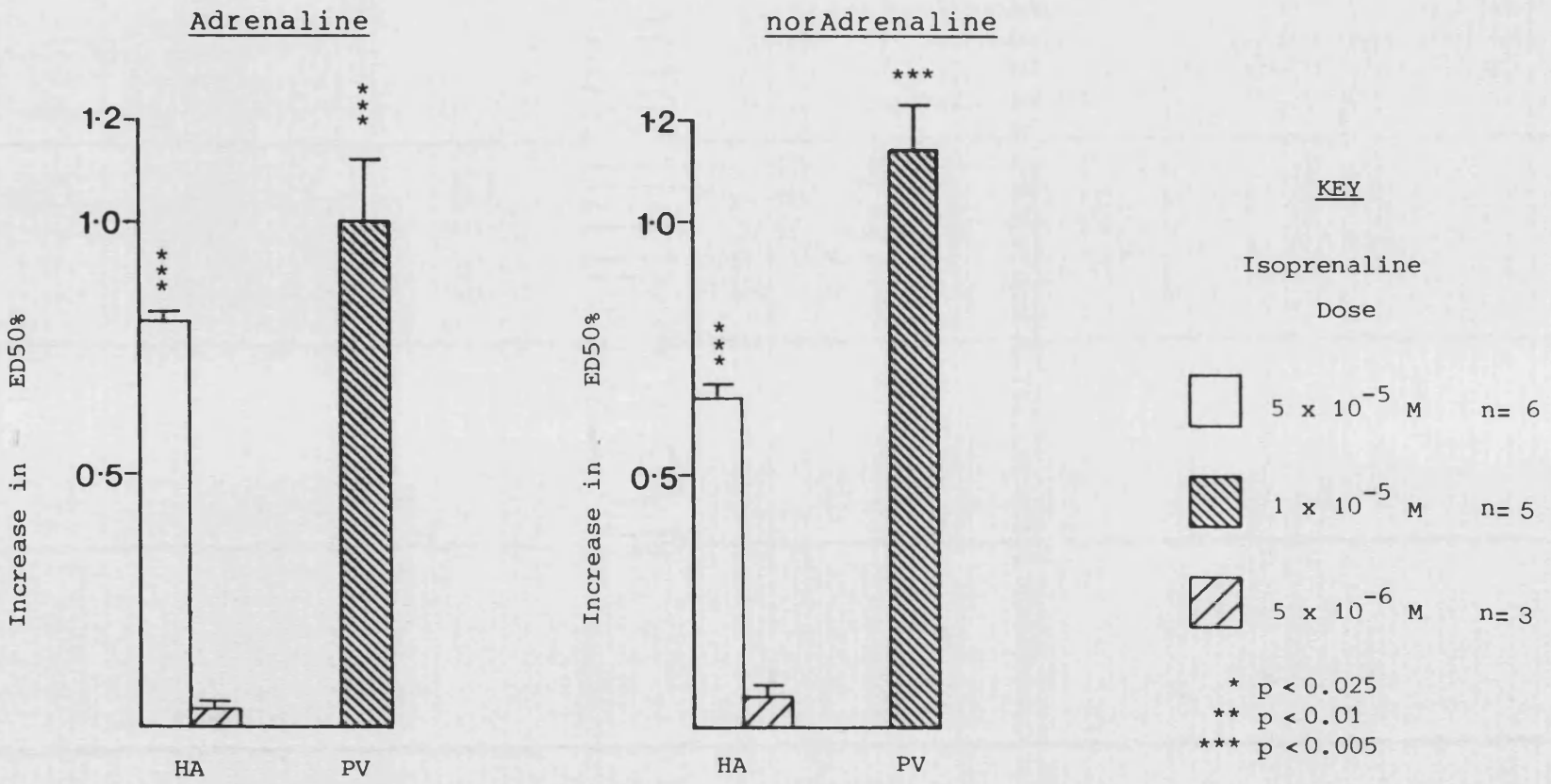
"X" = the difference in ED50% of curves "a" and "b".

This indirect method allows the characterisation of the β-adrenoreceptor activity in both the hepatic artery and the portal vein. It was also possible using selective β-adrenoreceptor blockers to catagorise the receptors in to β_1 and β_2 subtypes.

Figure 3.2.11 shows the effect of the β-adrenoreceptor agonist isoprenaline on the log dose response curves of adrenaline and noradrenaline. Isoprenaline is effective in the hepatic artery, (at 5×10^{-5} M $p<0.005$), and the portal vein,(at 1×10^{-5} M $p<0.005$), against both vasoconstrictors. Isoprenaline is more effective in the portal vein than in the hepatic artery against both adrenaline and noradrenaline. This may reflect a larger population of β-adrenoreceptors in the portal vein than in the hepatic artery.

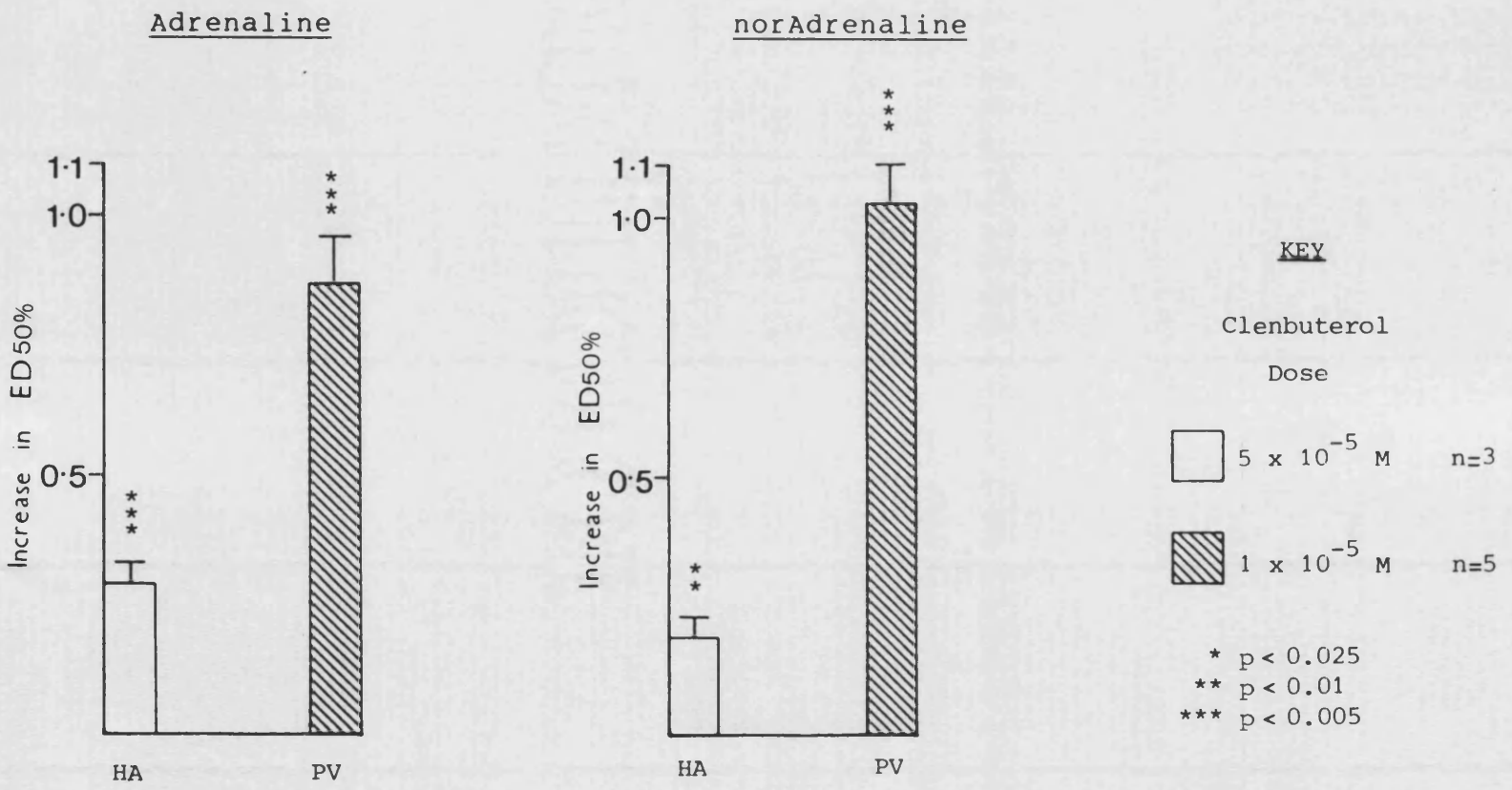
The results obtained during infusions of clenbuterol are presented in figure 3.2.12. Clenbuterol is a specific β_2-adrenoreceptor agonist, (Waldeck et al., 1985). The agonist is effective on adrenaline and noradrenaline induced vasoconstriction in both the hepatic artery, (at 5×10^{-5} M $p<0.01$) and the portal vein, (at 1×10^{-5} M $p<0.005$). The histogram shows a large difference in the effectiveness of the agonist between the two vessels. Clenbut-

Figure 3.2.11. The Effect of Hepatic Artery and Portal Vein Infusions of Isoprenaline on the ED50% of Adrenaline and norAdrenaline



Bars are means + sem. Data is given in Appendixes 16 and 17

Figure 3.2.12. The Effect of Hepatic Artery and Portal Vein Infusions of Clenbuterol on the ED50% of Adrenaline and norAdrenaline



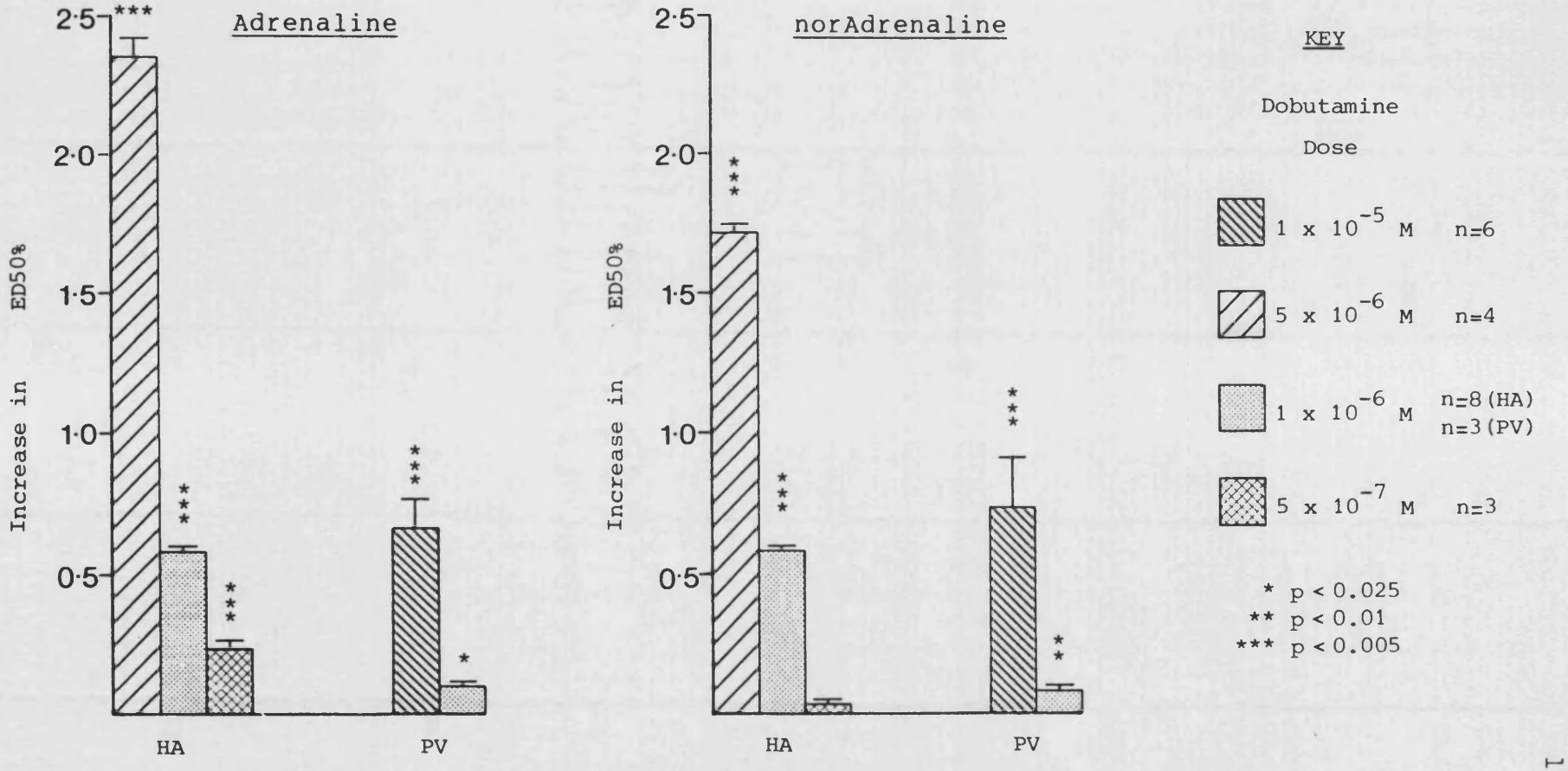
Bars are means + sem. Data is given in Appendixes 18 and 19

erol is more effective in the portal vein and since it is a selective β_2-adrenoreceptor agonist, the portal vein must contain a predominance of β_2-adrenoreceptors.

Figure 3.2.13 shows the results obtained when the β-adrenoreceptor agonist Dobutamine was infused. Although this compound is considered to be a selective β_1-adrenoreceptor agonist it has been demonstrated to have both β_2 and α-adrenoreceptor agonist activity, (Ruffolo et al., 1981, 1983; Maccarrone et al., 1984; Kenakin, 1981). The major action of the drug is still thought to be mediated via β_1-adrenoreceptors. Dobutamine was found to be effective against adrenaline and noradrenaline vasoconstriction in the hepatic artery, (at 1×10^{-6} M $p<0.005$) and the portal vein, (at 1×10^{-5}M $p<0.005$). The histogram indicates that dobutamine is more effective in the hepatic artery than the portal vein. Since dobutamine has a higher affinity for β_1-adrenoreceptors the results suggest that β_1-adrenoreceptors predominate in the hepatic artery.

The results obtained using the three β-adrenoreceptor agonists indicate a degree of heterogeneity between the hepatic artery and the portal

Figure 3.2.13. The Effect of Hepatic Artery and Portal Vein Infusions of Dobutamine on the ED50% of Adrenaline and norAdrenaline



Bars are means + sem. Data is given in Appendixes 20 and 21

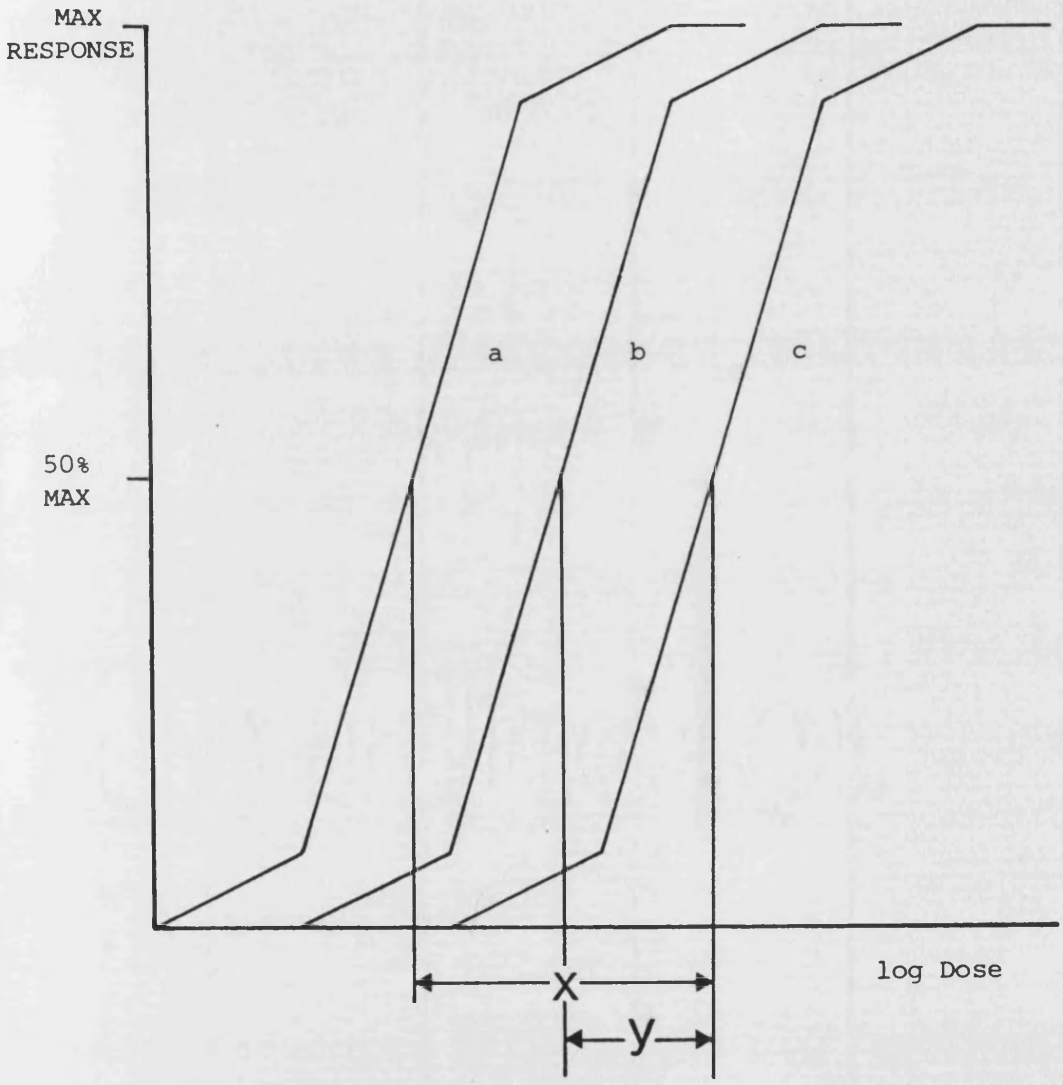
vein with respect to their β-adrenoreceptor populations. β_1-adrenoreceptors predominate in the hepatic artery whereas β_2-adrenoreceptors predominate in the portal vein vasculature. The results of this study contradict the findings of Richardson et al. (1977d) who showed that β_2-adrenoreceptors predominate in the hepatic artery of the dog. However, species differences may be responsible for this discrepancy. The results are consistent with those of Koo et al. (1979a) and Reilly et al. (1981) who showed a predominance of β_2-adrenoreceptors in the portal vein vasculature on the rat.

Since the measurement of β-adrenoreceptor activity was carried out using an indirect method, confirmation was needed to show that β-adrenoreceptor agonism not α-adrenoreceptor antagonism was responsible for the shift in vasoconstrictor ED50% seen during infusions of isoprenaline, clenbuterol and dobutamine. The specificity of the shift in constrictor ED50% due to infusions of the three β-adrenoreceptor agonists was therefore assessed using β-adrenoreceptor blocking drugs. The effectiveness of these drugs on β-adrenoreceptor agonism was measured using shifts in ED50% of adrenaline and noradrenaline log dose response curves during

β-adrenoreceptor agonist infusion with and without β-adrenoreceptor blockade. Figure 3.2.14 is a schematic representation of the log dose curves used to calculate the effectiveness of the β blockers. The difference between the curve "a" and the curve "c", "X" represents the shift in ED50% of the constrictor due to β-adrenoreceptor agonist infusion. The shift from curve "c" to "b", shown as "Y", represents the degree of β-adrenoreceptor antagonism due to infusions of β blockers. The degree of β blockade is expressed as a % reduction this being (Y/X) x 100. The greater the the value of the % reduction the greater the β-adrenoreceptor blockade. All three β-adrenoreceptor agonists were tested using all five β blockers. All the β blockers used show varying degrees of β_1 and β_2 -adrenoreceptor selectivity. Atenolol, metoprolol and oxprenolol are relatively selective β_1 -adrenoreceptor blockers. Propranolol shows a mixed β_1 and β_2 antagonism with β_1 predominance. ICI 118 551 is a selective β_2 blocker, (Arnold et al., 1985 and Bilski et al. 1983). All the β blockers were infused in concentrations which had been shown to have no direct effect on adrenaline or noradrenaline induced vasoconstriction in either vessel,(tables 3.2.1, 3.2.2, 3.2.3).

Figure 3.2.14.

A Schematic Representation to Show the Calculation of The Percentage Reduction on Adrenaline and norAdrenaline ED50%, in the Presence of β-Adrenoreceptor Agonism and Antagonism



"a"= the normal constrictor log dose response curve.

"c"= the constrictor log dose response curve in the presence of β-adrenoreceptor agonism.

"b"= the constrictor log dose response curve in the presence of β-adrenoreceptor agonism and β blockade.

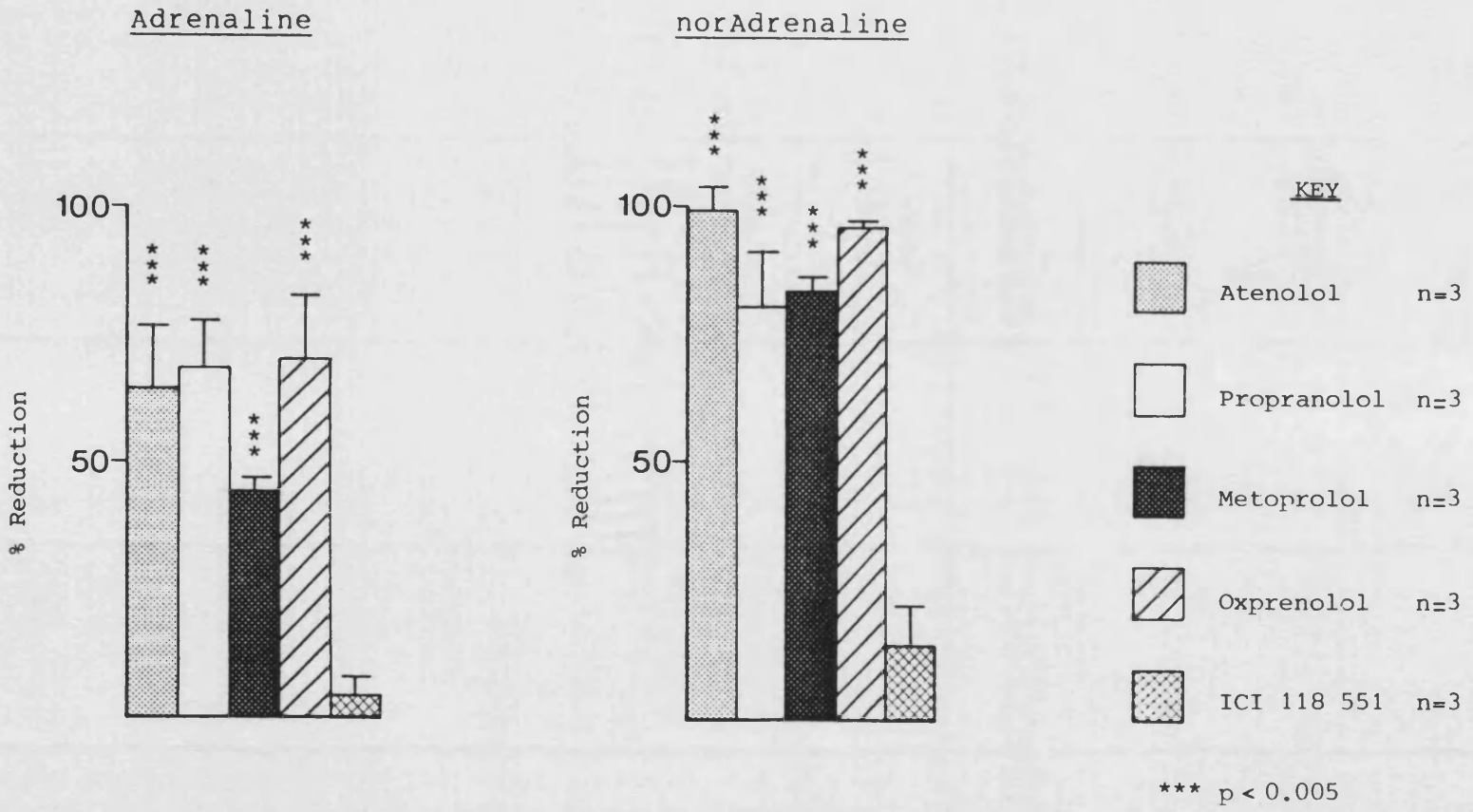
"X"= the difference in ED50% of curves "a" and "c".

"Y"= the difference in ED50% of curves "c" and "b"

% Reduction= (Y/X) x 100

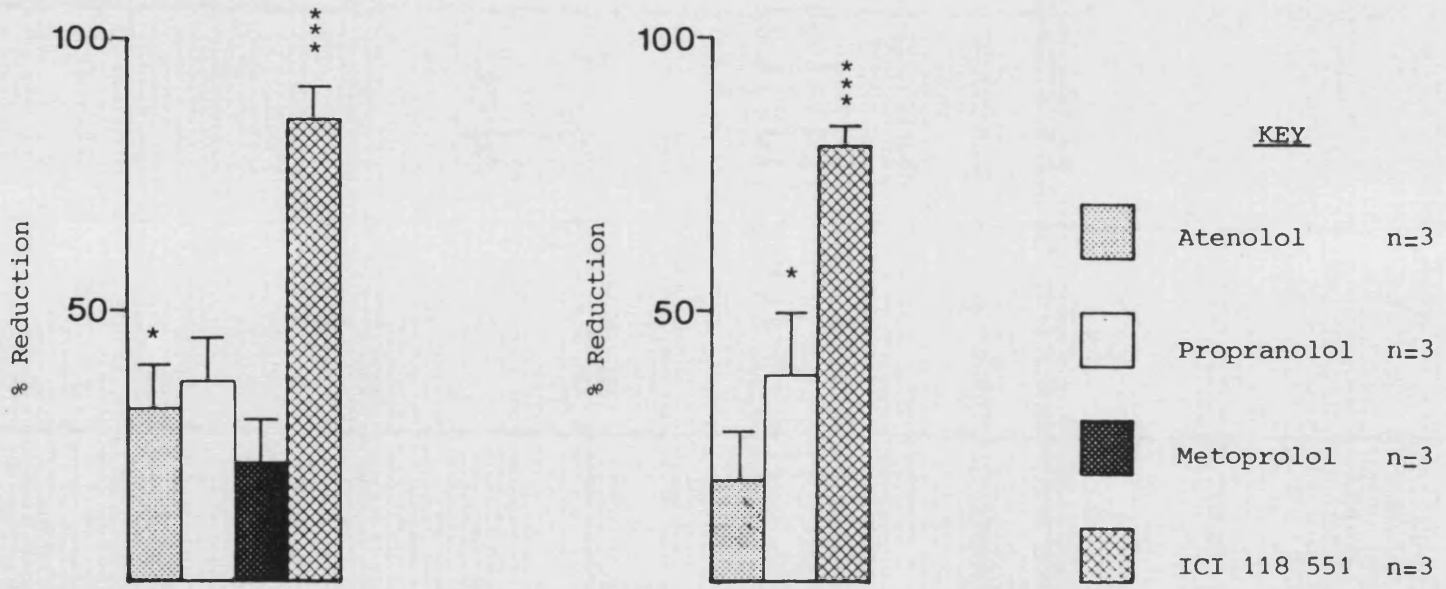
Figure 3.2.15 shows the results of the β-adrenoreceptor blocking drugs on isoprenaline activity in the hepatic artery. Atenolol, propranolol, metoprolol and oxprenolol show a statistically significant ($p<0.005$) blockade of isoprenaline induced shifts in ED50% of adrenaline and noradrenaline. Using noradrenaline as the constrictor the β blockers produced almost complete blockade of isoprenaline. This shows that isoprenaline was indeed causing its effects in the hepatic artery by acting on β-adrenoreceptors. The β_2-adrenoreceptor blocking drug ICI 118 551 was ineffective at antagonising isoprenaline in the hepatic artery. The relatively selective β_1-adrenoreceptor blocking drugs are more effective in this vessel and suggest that the hepatic artery contains β-adrenoreceptors predominantely of the β_1 subtype. The results of a similar experiment carried out on the portal vein are given in figure 3.2.16. Here it would appear that the β_2 selective blocker ICI 118 551 is more effective than the relatively selective β_1 blocking drugs atenolol, metoprolol and propranolol at antagonising isoprenaline. The blockade due to ICI 118 551 was almost 100% and indicates that effects of isoprenaline are due to its action on β-adrenoreceptors. The ineffective-

Figure 3.2.15. The Effect of 5 X 10^{-6} M Infusions of Various β Blockers on the Shift in ED50% of Adrenaline and norAdrenaline due to Hepatic Artery Infusions of 5 X 10^{-5} M Isoprenaline.



Bars are means + sem. Data is given in Appendix 22.

Figure 3.2.16. The Effect of 1 X 10^{-5} M Infusions of Various β Blockers on the Shift in ED50% of Adrenaline and norAdrenaline due to Portal Vein Infusions of 1 X 10^{-5} M Isoprenaline.

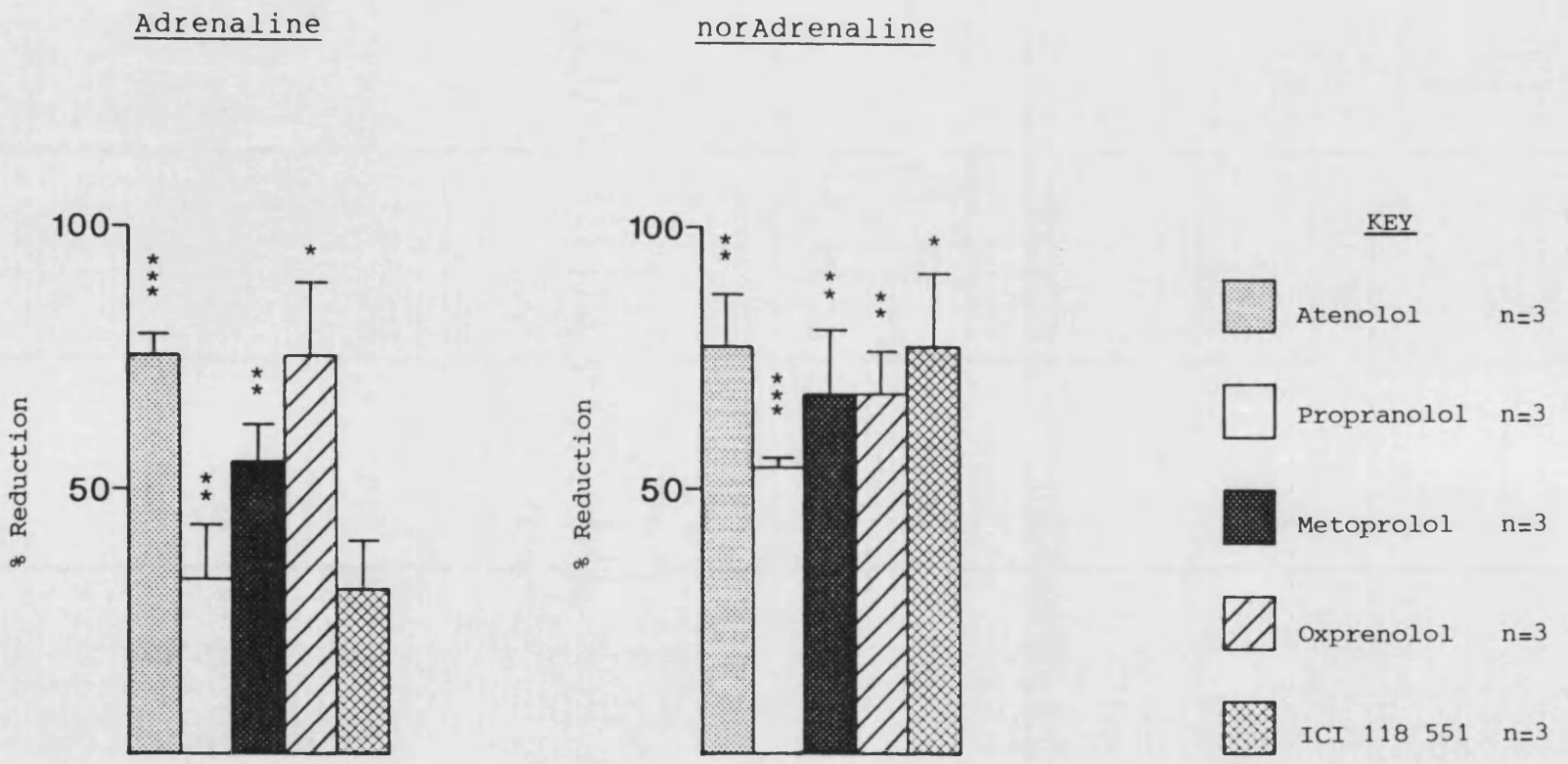


Bars are means + sem. Data is given in Appendix 23.

ness of the relatively β_1 selective blockers in the portal vein and the effective blockade by ICI 118 551 indicate a predominance of β_2-adrenoreceptors in this vessel.

Figure 3.2.17 shows the results of experiments to study the effects of β blockers on the activity of the β_2 selective agonist clenbuterol in the hepatic artery. These show the that both β_1 and β_2 selective adrenoreceptor blocking drugs are similar in their ability to antagonise clenbuterol activity. It was shown in figure 3.2.12 that although clenbuterol produces a significant shift ($p<0.01$) in ED50% of both constrictors in both vessels it is more effective in the portal vein. Also from the evidence in figures 3.2.12, 3.2.13, and 3.2.14 β_1-adrenoreceptors predominate in the hepatic artery. Clenbuterol although being a selective β_2-adrenoreceptor agonist probably possesses some β_1-adrenoreceptor activity. The β-adrenoreceptor activity of clenbuterol recorded in the artery could be a combination of weak stimulation of a large number of β_1-adrenoreceptors and stimulation of a smaller number of β_2-adrenoreceptors. Since both selective β_1 and β_2 blockers are equally effective at antagonising clenbuterol in the artery it would seem that this explan-

Figure 3.2.17. The Effect of 5 X 10^{-6} M Infusions of Various β Blockers on the Shift in ED50% of Adrenaline and norAdrenaline due to Hepatic Artery Infusions of 5 X 10^{-5} M Clenbuterol.

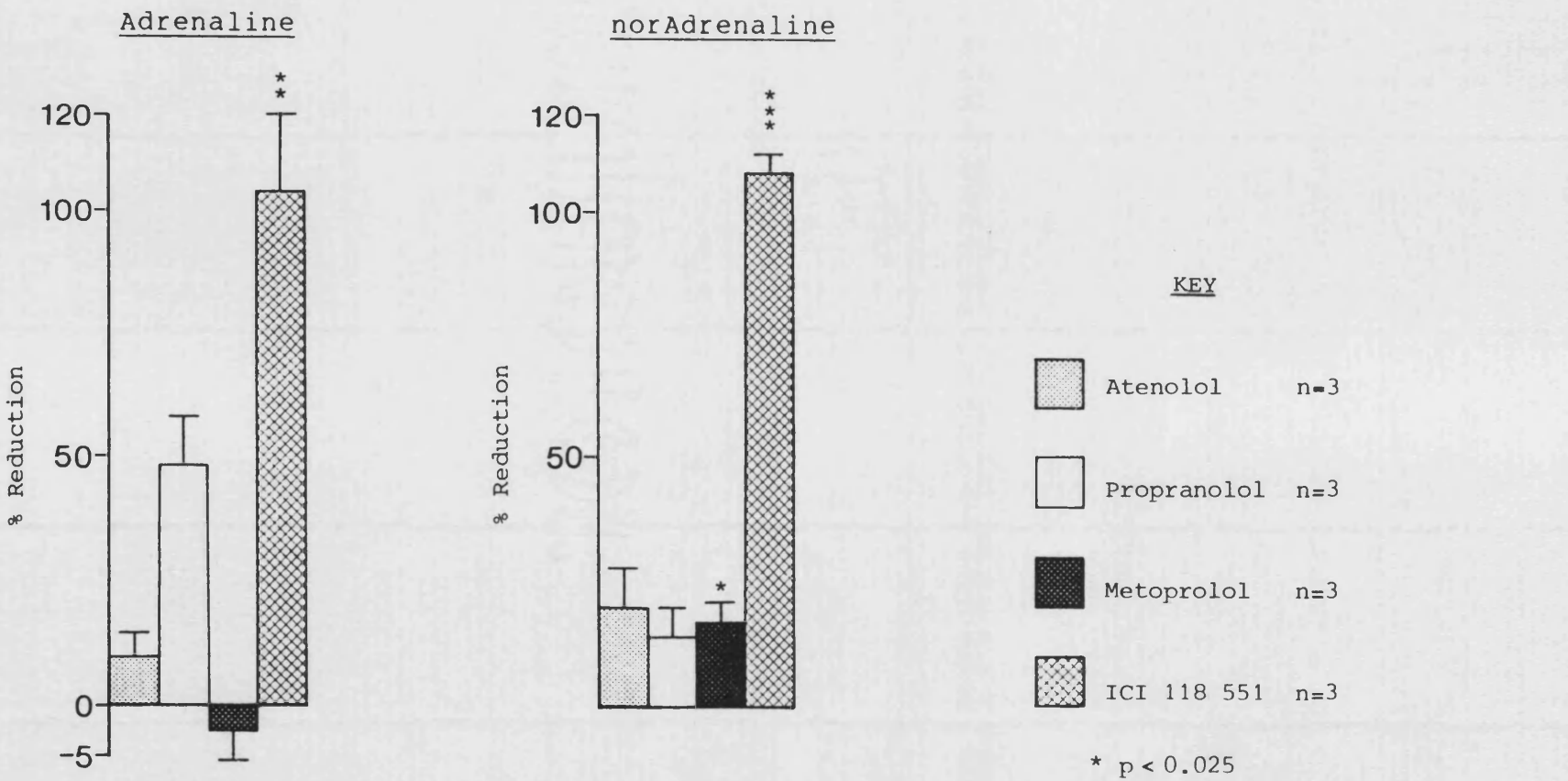


Bars are means + sem. Data is given in Appendix 24.

ation of its action is probable. The results again indicate β_1-adrenoreceptor predominance in the hepatic artery. Figure 3.2.18 shows the effect of the β blockers on clenbuterol activity in the portal vein. In this vessel the blocker ICI 118 551 is effective at antagonising clenbuterol and shows that action of clenbuterol is due to β-adrenoreceptor agonism and not α-adrenoreceptor antagonism. The ineffectiveness of the relatively selective β_1-adrenoreceptor blockers on clenbuterol indicate that the portal vein contains a predominance of β_2-adrenoreceptors.

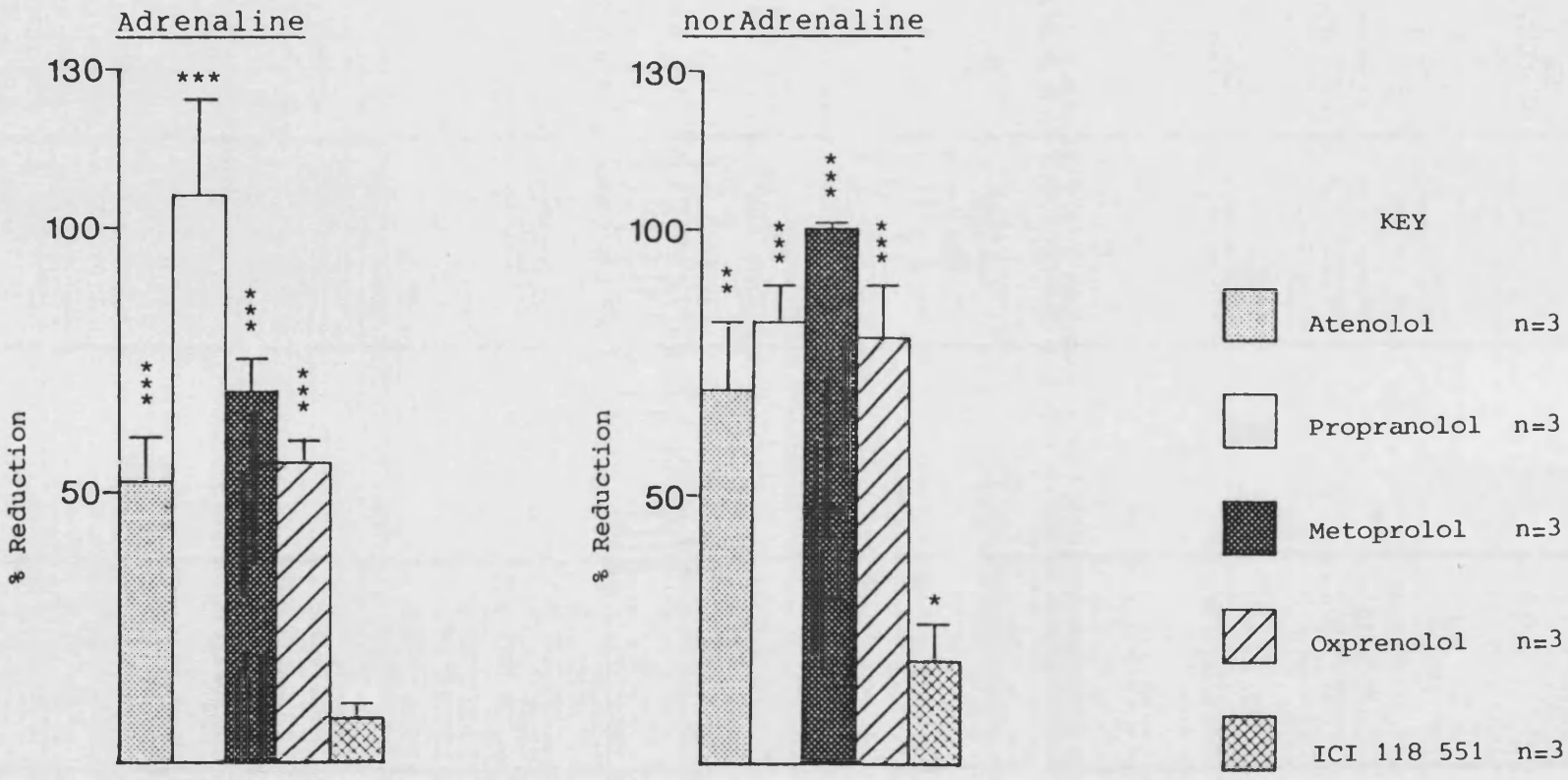
Antagonism of dobutamine β-adrenoreceptor agonism was also studied and the results for the hepatic artery are given in figure 3.2.19. The relatively selective β_1 blockers atenolol, propranolol, metoprolol and oxprenolol produce effective blockade in the artery. This shows that effects of dobutamine are due to β-adrenoreceptor agonism and not α-adrenoreceptor antagonism. The β_2 selective blocker ICI 118 551 is not very effective at antagonising dobutamine in the hepatic artery and this together with the effectiveness of the β_1 selective blockers indicates that β_1-adrenoreceptors predominate in the hepatic artery. Figure 3.2.20 shows the effect of β blockers on dobutamine

Figure 3.2.18. The Effect of 1 X 10^{-5} M Infusions of Various β Blockers on the Shift in ED50% of Adrenaline and norAdrenaline due to Portal Vein Infusions of 1 X 10^{-5} M Clenbuterol.



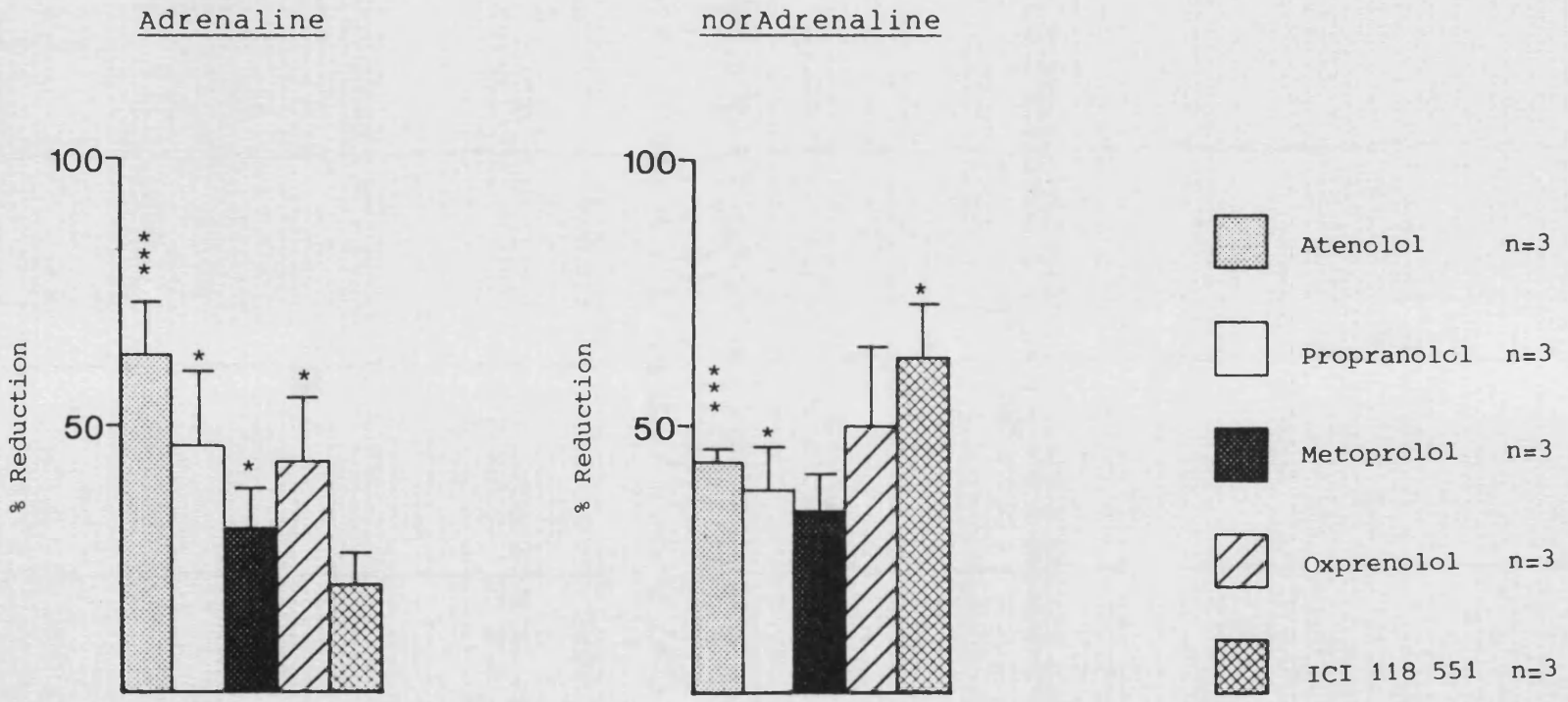
Bars are means + sem. Data is given in Appendix 25.

Figure 3.2.19. The Effect of 5 X 10^{-6} M Infusions of Various β Blockers on the Shift in ED50% of Adrenaline and norAdrenaline due to Hepatic Artery Infusions of 1 X 10^{-6} M Dobutamine.



Bars are means + sem. Data is given in Appendix 26.

Figure 3.2.20. The Effect of 1 X 10^{-5} M Infusions of Various β Blockers on the Shift in ED50% of Adrenaline and norAdrenaline due to Portal Vein Infusions of 1 X 10^{-5} M Dobutamine.



Bars are means + sem. Data is given in Appendix 27.

in the portal vein. All five blockers are similar in their ability to antagonise dobutamine. As shown in figure 3.2.13 dobutamine is less effective in the portal vein than the hepatic artery. This seems to point to a predominance of β_2-adrenoreceptors in the portal vein. Dobutamine may show a weak stimulation of β_2-adrenoreceptors and a larger affinity for a smaller number of β_1-adrenoreceptors in the portal vein. If this is the case then infusions of β_1 and β_2 selective blockers would antagonise dobutamine to the same degree. The results are thus, consistent with the predominance of β_2-adrenoreceptors in the portal vein.

In summary the results from experiments to study the β-adrenoreceptor population in the liver vasculature have shown:-

i) That the β-adrenoreceptor agonists isoprenaline, clenbuterol and dobutamine all simulate β-adrenoreceptor mediated dilation in the hepatic artery and the portal vein.

ii) The selective β_2-adrenoreceptor agonist clenbuterol is more effective in the portal vein whilst the β_1-adrenoreceptor agonist dobutamine is more effective in the hepatic artery.

iii) The relatively selective β_1-adrenoreceptor blocking drugs propranolol, atenolol, metoprolol and oxprenolol are in general more effective in the hepatic artery. The β_2-adrenoreceptor blocking drug ICI 118 551 is more effective in the portal vein.

iv) The results point to a degree of heterogeneity in the β-adrenoreceptor population in the hepatic artery and the portal vein. The hepatic artery possessing a predominance of β_1-adrenoreceptors and the portal vein a predominance of β_2-adrenoreceptors.

(d) Interactions between the hepatic artery and the portal vein

It was observed in the isolated perfused rat liver experiments that infusions of adrenaline or noradrenaline into one vessel may produce an increase in perfusion pressure not only in the infusion vessel but also the other vessel. Experiments were carried out in the perfused rat liver to determine the extent of this interaction between the two vessels. It was found that infusions of noradrenaline into the portal vein could produce a dose related increase in pressure not only in the portal vein but also in the hepatic

artery. A log dose response curve could be constructed for both vessels during portal vein infusions of noradrenaline. Infusions of high concentrations of noradrenaline in to the hepatic artery were occasionally able to produce an increase in portal vein pressure. A log dose response curve of increase in portal vein pressure against log hepatic artery noradrenaline dose could not be obtained. The concentration of hepatic artery infusions of noradrenaline required to produce portal vein constriction were so high that frequently the hepatic artery became permanently constricted.

The increases in pressure seen in the hepatic artery during portal vein infusions of noradrenaline could be due to constriction of the hepatic veins. However, if the liver output vessels are constricted during portal vein noradrenaline infusions the increase in the hepatic artery and portal vein perfusion pressures should be similar. An alternative explanation is that there are presinusoid connections between the hepatic artery and the portal vein. Studies by Nopanitaya et al., (1978), Hase et al., (1966), Geumei, (1969a) and Grisham et al. (1981) suggest the presence of such connections. Also, experiments by Green et al., (1959), Richardson et al., (1978a,

1978b), Hirsch et al. (1976) in dogs and Lautt et al. (1984) in cats have shown that infusions of vasoactive agents into one vessel may affect the other other liver input vessel.

The log dose response curves obtained in experiments with the isolated perfused rat liver were sigmoidal and allowed the calculation of ED50% values for each curve. The results are presented in table 3.2.4. The ED50% of noradrenaline induced vasoconstriction in the hepatic artery due to portal vein infusions is greater ($p<0.01$),than the ED50% in the hepatic artery during hepatic artery infusion and the portal vein ED50% during portal vein infusion. This suggests that portal vein access to the hepatic artery resistance vessels is small. Geumei (1969a), demonstrated the existence of unidirectional arterio-portal connections. Since hepatic artery infusions of noradrenaline only occasionally produced an increase in portal vein perfusion pressure, any unidirectional arterio-portal connections as seen by Geumei (1969a), must bypass the portal vein resistance vessels. The results of table 3.2.4 do however, indicate that connections allowing flow from the portal may exist as portal vein noradrenaline infusions produce vasoconstriction in the hepatic artery.

Table 3.2.4. The ED50% of norAdrenaline Induced vasoconstriction when infused via the Hepatic Artery or the Portal Vein

Route of Infusion	ED50% of Constriction ± s.e.m.	
	Hepatic Artery n=5	Portal Vein n=5
Hepatic Artery	$2.57 \pm 0.14 \times 10^{-7}$ M	a
Portal Vein	$6.82 \pm 1.50 \times 10^{-6}$ M	$1.12 \pm 0.5 \times 10^{-6}$ M

"a" indicates that it was not possible to produce a complete dose response could not be produced and hence an ED50% could not be calculated.

Values are means ± standard error of the mean.

The shift in log dose response curves was investigated in each vessel during hepatic artery and portal vein infusions of 1 X 10^{-6} M phentolamine. The results are given in table 3.2.5. During hepatic artery phentolamine infusions there was a significant shift ($p<0.01$), in all three log dose response curves. The greatest shift was to the hepatic artery log dose response curve during noradrenaline infusion into the same vessel. The lowest shift was seen in the log dose response curve in the portal vein. The hepatic artery log dose response curve due to portal vein infusions of noradrenaline showed an intermediate shift. If perfusate from the portal vein perfusing the hepatic artery passed through the the same vessels as that perfusate entering by the hepatic artery, it would be expected that phentolamine would shift the log dose response curves in the hepatic artery by similar amounts. Since this does not occur the portal vein perfusate passing into the hepatic artery system may perfuse vessels not normally highly perfused by the hepatic artery.

During portal vein infusions of phentolamine, both hepatic artery log dose response curves show a lower shift in ED50% than those seen in the portal vein. This indicates that portal vein access to

Table 3.2.5. The Effect of Portal Vein and Hepatic Artery Infusions of 1 X 10^{-6} M Phentolamine on the ED50% of norAdrenaline Vasoconstriction

Phentolamine Infusion Route	norAdrenaline Infusion Route	CHANGE IN ED50%	
		Hepatic Artery n=5	Portal Vein n=5
HA	HA	1.00 ± 0.08 ***	a
	PV	0.66 ± 0.06 ***	0.43 ± 0.05 **
PV	HA	0.31 ± 0.05 **	a
	PV	0.27 ± 0.05 **	0.71 ± 0.07 ***

Values are changes in ED50% ± Standard error of the mean

"a" indicates that a complete dose response curve could not be produced.

The Shift in ED50% was compared using the related "t" test.

** $p < 0.01$
*** $p < 0.005$

the perfusate in the hepatic resistance vessels is small.

The results of the experiments to investigate vasculature interactions between the hepatic artery and the portal vein have shown that connections from the portal vein to the hepatic arterial resistance vessels exist but their number is small. Also the hepatic artery vessels to which the portal vein has access may not be those normally perfused by the hepatic artery.

3) EFFECT OF ADRENORECEPTOR MEDIATED INCREASES IN PERFUSION PRESSURE ON LIGNOCAINE METABOLISM

(a) Effect on Lignocaine Extraction ratio.

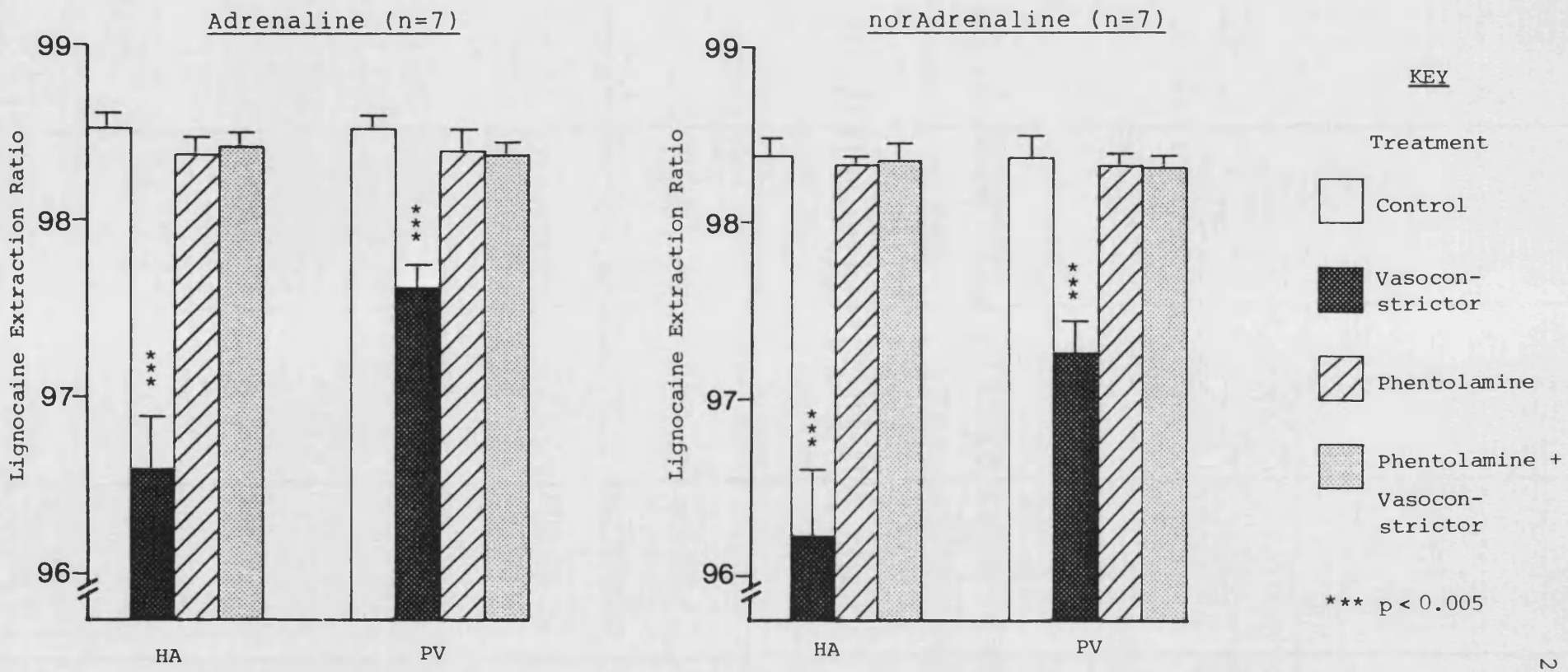
From the results obtained in the isolated perfused rat using adrenoreceptor agonists and antagonists it was intended to produce a system where perfusion pressure could be increased and decreased at constant HA:PV flow ratio. However, it was only possible to produce direct vasoconstriction in the hepatic vessels so only increases in perfusion pressure could be investigated. The preparation was found to be maximally dilated and vasodilators were without effect. Only the effects of increases in perfusion pressure on the metabolism of lignocaine at a constant HA:PV flow ratio were investigated. Both adrenaline and noradrenaline infusions were used as vasoconstrictors to increase perfusion pressure during lignocaine perfusion. Although experiments by Dixon et al. (1964), had shown in rat liver microsomes that incubation for short periods with noradrenaline did not effect hydroxylation or dealkylation, the possibility existed that adrenaline or noradrenaline could inhibit the enzymes responsible for lignocaine

metabolism. In order to overcome this problem phentolamine was infused with the vasoconstrictors to prevent pressure increases whilst still allowing adrenaline and noradrenaline to exert any enzyme inhibition. Phentolamine infused alone did not change perfusion pressure and was used as a control for phentolamine enzyme inhibition.

Perfusions were carried out at the HA:PV flow ratio of 2.5:7.5 (as outlined in section 8d of materials and methods). A concentration of constrictor was infused into each vessel to give a 70% to 80% maximal increase in perfusion pressure. This increased hepatic artery pressure approximates to that seen in experiments where the hepatic artery flow rate is 5.0 ml/min. Although increases in portal vein pressure are negligible when flow is increased in the vessel, the effect of increases in portal vein perfusion pressure were also investigated.

Figure 3.3.1 shows the effect of increases in perfusion pressure on the extraction ratio of lignocaine. Only where livers are perfused with vasoconstrictor alone is there an increase in perfusion pressure. In all cases where perfusion pressure is raised in the hepatic artery and the portal vein, the extraction ratio of lignocaine is reduced, ($p<0.05$).

Figure 3.3.1. The Effect of Adrenaline and norAdrenaline Induced Increases in Perfusion Pressure on the Extraction Ratio of Lignocaine.



Bars are means + sem. Data is given in Appendix 28.

The decrease is more pronounced in the hepatic artery than in the portal vein. The reduction was not due to enzyme inhibition as when adrenaline or noradrenaline are co-infused with phentolamine no reduction in lignocaine extraction ratio is recorded. Phentolamine infusion alone also had no effect on lignocaine extraction ratio confirming that the decrease in extraction ratio was due to an increase in perfusion pressure. The extraction ratios of lignocaine seen during increases in hepatic artery perfusion pressure (96.59 for adrenaline and 96.25 for noradrenaline), are similar to that seen when the hepatic artery flow is 5ml/min (96.07, see fig 3.1.1). The increase in perfusion pressure could account for the decrease in extraction ratio seen during increases in hepatic artery flow contribution. Although from figure 3.3.1 a reduction in portal vein perfusion pressure would result in an increase in extraction ratio, portal vein perfusion pressure remains relatively constant during changes in flow. Thus, during changes in the HA:PV flow ratio there would be no compensatory increase in extraction ratio due to decreases in portal perfusion pressure, to balance the decrease in extraction ratio which occurs during increases in hepatic artery perfusion pressure. Since hepatic artery perfusion

pressure is related to flow and there is a relationship between hepatic artery flow and lignocaine extraction ratio, the changes in hepatic artery perfusion pressure can account for the decreased lignocaine extraction ratio seen during increased hepatic artery flow.

Lautt et al. (1976) in rats carried out an experiment in which he measured lignocaine extraction ratio during hepatic nerve stimulation. The nerve stimulation caused an increase in both hepatic artery pressure, (from 121 mmHg to 134 mmHg) and portal vein pressure,(from 5 mmHg to 8 mmHg) The extraction ratio of lignocaine was reduced from 33.5% to 29.7% during nerve stimulation. The reduction in extraction ratio was not found to be significant. However, in the cat there is an reduction of vasoconstriction during continuous nerve stimulation. The changes in perfusion pressure also are small compared with those induced in the present study. These two factors could account for the lack of a significant drop in lignocaine extraction ratio during nerve stimulation in cats.

There are several possible explanations to account for the effect of increased perfusion pressure on lignocaine extraction ratio.

i) Arterio-venous shunting.

This involves the diversion of perfusate from its normal route through the liver via non metabolic pathways to the venous system. The diversion will result in an increase in venous concentration of any perfused drug and a decrease in apparent extraction ratio. Wood et al. (1982), in rats and Groszmann et al. (1977), in humans have shown that shunting occurs in normal livers. Ahmad (1982), has shown that shunting is larger during hepatic artery perfusion of the liver. This may well be a consequence of increase in hepatic artery perfusion pressure and could account for the decrease in lignocaine extraction ratio seen during increases in perfusion pressure.

ii) Changes in the space of Disse.

Ahmad (1982), also showed that the apparent space of Disse is greater in rats when the liver is perfused via the hepatic artery. An increase in the space of Disse may result in a decreased lignocaine extraction ratio. An increase in the space of Disse may occur due to an increase in hepatic artery perfusion pressure during increased flow but it is difficult to see a mechanism by which this may occur. It is more likely that the results of Ahmad (1982) reflect an inherent difference in the space of Disse

between the two supplies.

iii) Changes in fenestral size.

Another possible explanation for the decrease in lignocaine extraction ratio during increases in perfusion pressure comes from experiments by Wisse et al. (1980, 1983). He found that the fenestrations of the epithelial cells can constrict when exposed to noradrenaline. This decrease in fenestral diameter could result in a reduction in the rate of passage of drug through the sinusoidal epithelium. The contact between drug molecules and the surface of the parenchymal cells would be reduced and result in a reduction of the extraction ratio of the drug. The reduction in fenestral size could account for the results seen during increases in perfusion pressure if phentolamine antagonises the action of the vasoconstrictor on changes in fenestral size. Thus, during co-infusion of vasoconstrictor and phentolamine the effect of adrenaline or noradrenaline on fenestral diameter is blocked. The reduction in extraction ratio of lignocaine may not be related to the ability of the vasoconstrictor to increase perfusion pressure but to its effect on fenestral size.

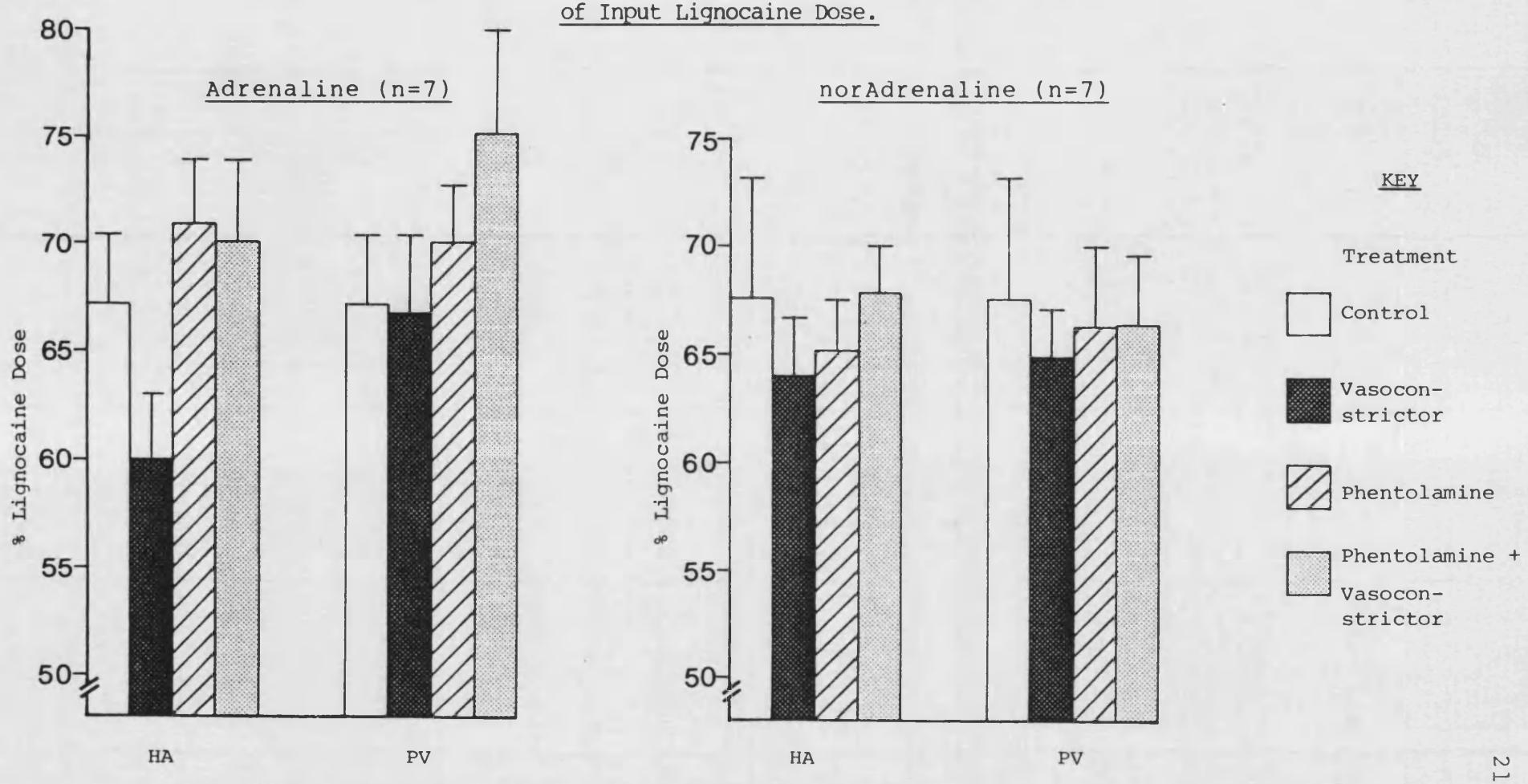
iv) Diversion of perfusate through different enzyme environments.

A fourth explanation to account for the effect of perfusion pressure on the extraction ratio of lignocaine involves the diversion of the perfusate through sinusoids with an unusual metabolic environment. Increases in perfusion pressure may open "static sinusoids", reported by Wakim et al. (1942), Bloch (1955) and McCuskey (1966) of direct flow through sinusoids not normally perfused by a particular supply, thus exposing incoming perfusate to a different metabolic environment. To investigate this possibility the metabolic profile of lignocaine was examined during changes in perfusion pressure at a constant HA:PV flow ratio of 2.5:7.5 ml/min.

(b) Effect of Changes in perfusion pressure on the metabolic profile of lignocaine.

Total recovery of lignocaine metabolites showed no significant change when pressure was raised in either the hepatic artery or the portal vein, (figure 3.3.2). Although a constant recovery of lignocaine would allow the expression of the results as a percent of dose for consistency with the results

Figure 3.3.2. The Effect of Adrenaline and norAdrenaline Induced Increases in Perfusion Pressure on the Combined Total Output Metabolite Concentration. Values are Expressed as Percentages of Input Lignocaine Dose.



Bars are means + sem. Data is given in Appendix 29.

presented previously, the metabolite concentrations were expressed as a percentage of combined total recovered metabolite concentration, (see figure 3.1.3 for explanation of result calculation). Figures 3.3.3, 3.3.4 and 3.3.5 show the results of the recovery of phase I metabolites during changes in perfusion pressure. Only where the vasoconstrictor was perfused alone was any increase in perfusion pressure recorded. The results show that no significant changes in metabolic profile of lignocaine occur during increases in hepatic artery or portal vein pressure. Figures 3.3.6, 3.3.7 and 3.3.8 show the effect of alterations in perfusion pressure on recovery of phase II lignocaine metabolites. The results indicate that no changes in phase II metabolism of lignocaine occur during changes in perfusion pressure in the hepatic artery or the portal vein.

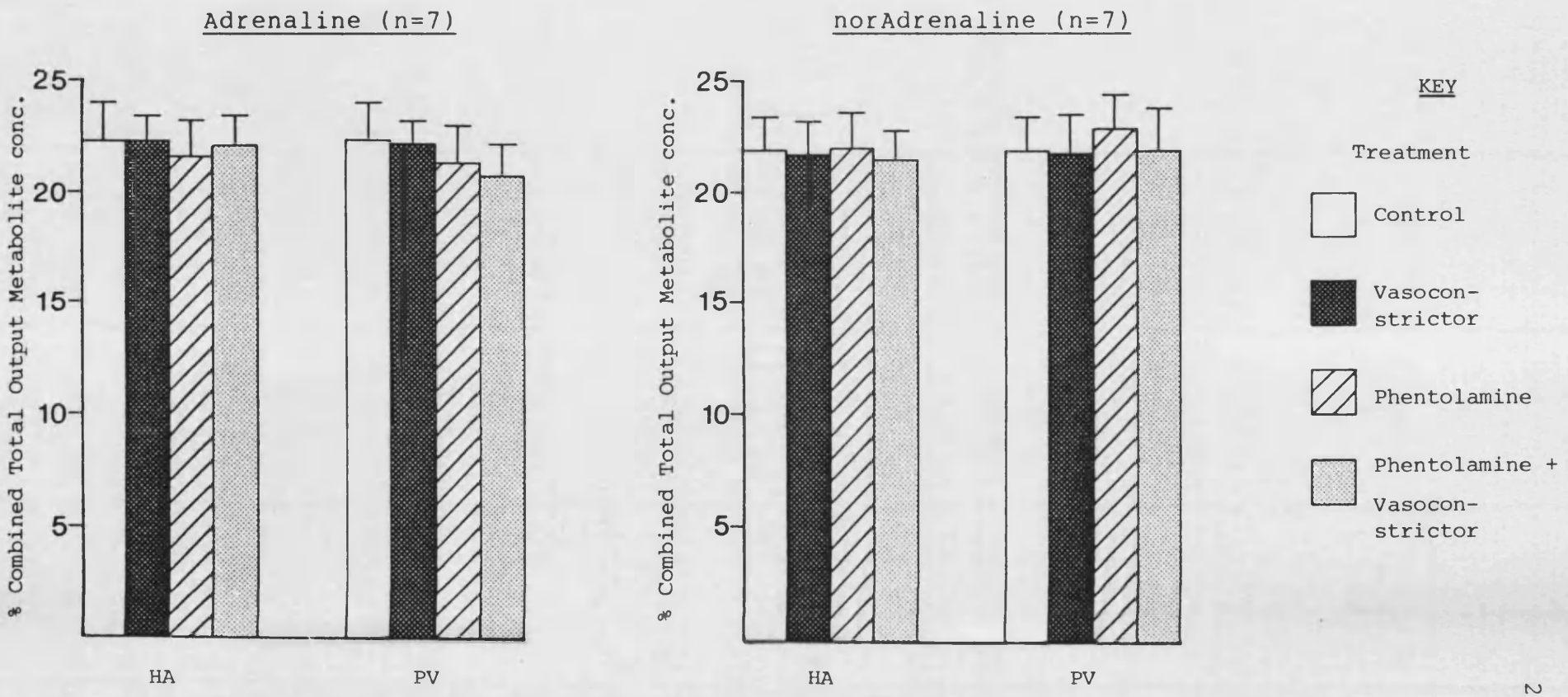
The studies carried out to investigate the effect of increases in perfusion pressure on the metabolism of lignocaine have shown the following:-

i) Increases in both hepatic artery and portal vein perfusion pressure due to infusions of adrenaline and noradrenaline reduce the extraction ratio of lignocaine. This reduction may be due to an increase in arterio-venous shunting, a decrease in fenestral diameter or a diversion of the perfusion

medium through "unusual" metabolic environments.

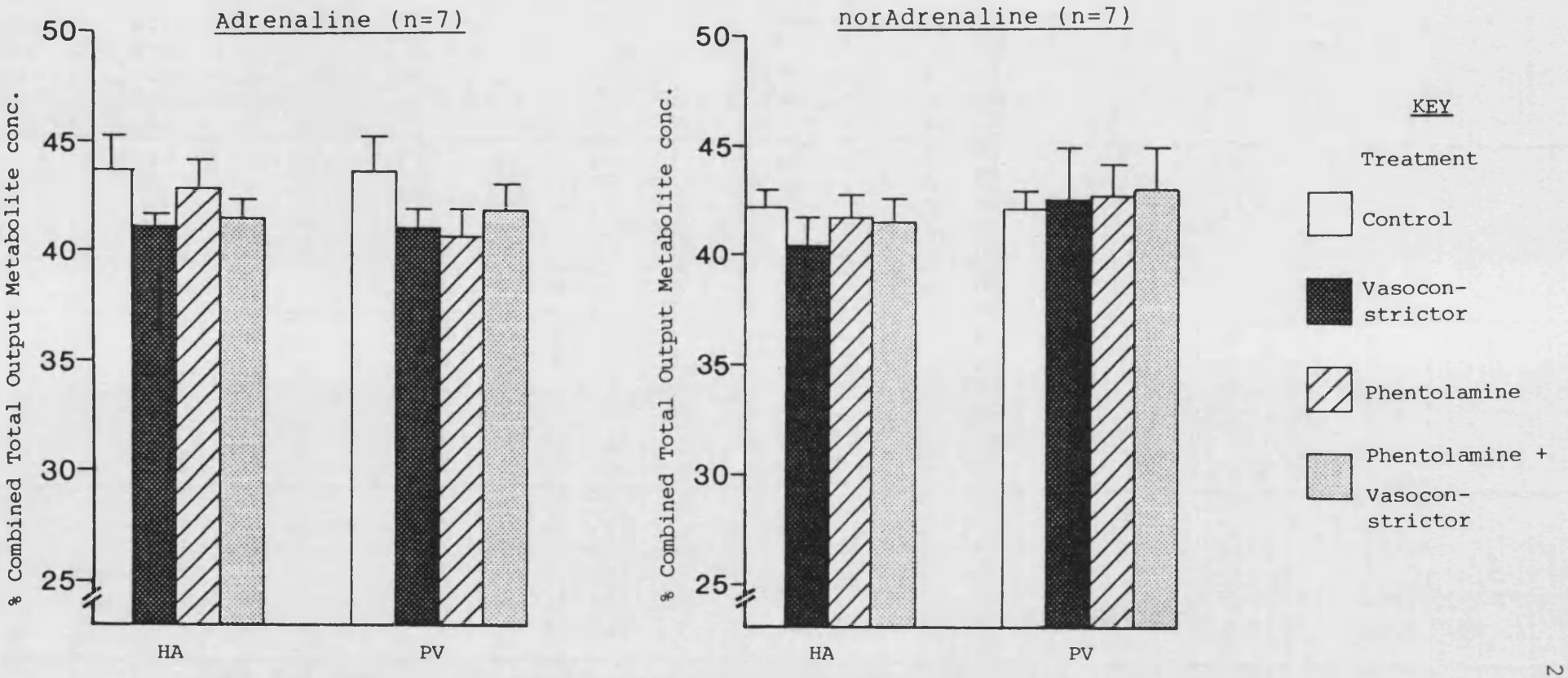
ii) The metabolism of the drug is not altered during changes in perfusion pressure. This shows that an increase in perfusion pressure is unlikely to divert perfusion medium through "unusual" metabolic channels. The possibility of shunting or reductions in fenestral diameter being responsible for the decrease in lignocaine extraction ratio still exists.

Figure 3.3.3. The Effect of Adrenaline and norAdrenaline Induced Increases in Perfusion Pressure on the Recovery of Total 3-OH Lignocaine. Values are Expressed as Percentages of Combined Total Output Metabolite Concentration.



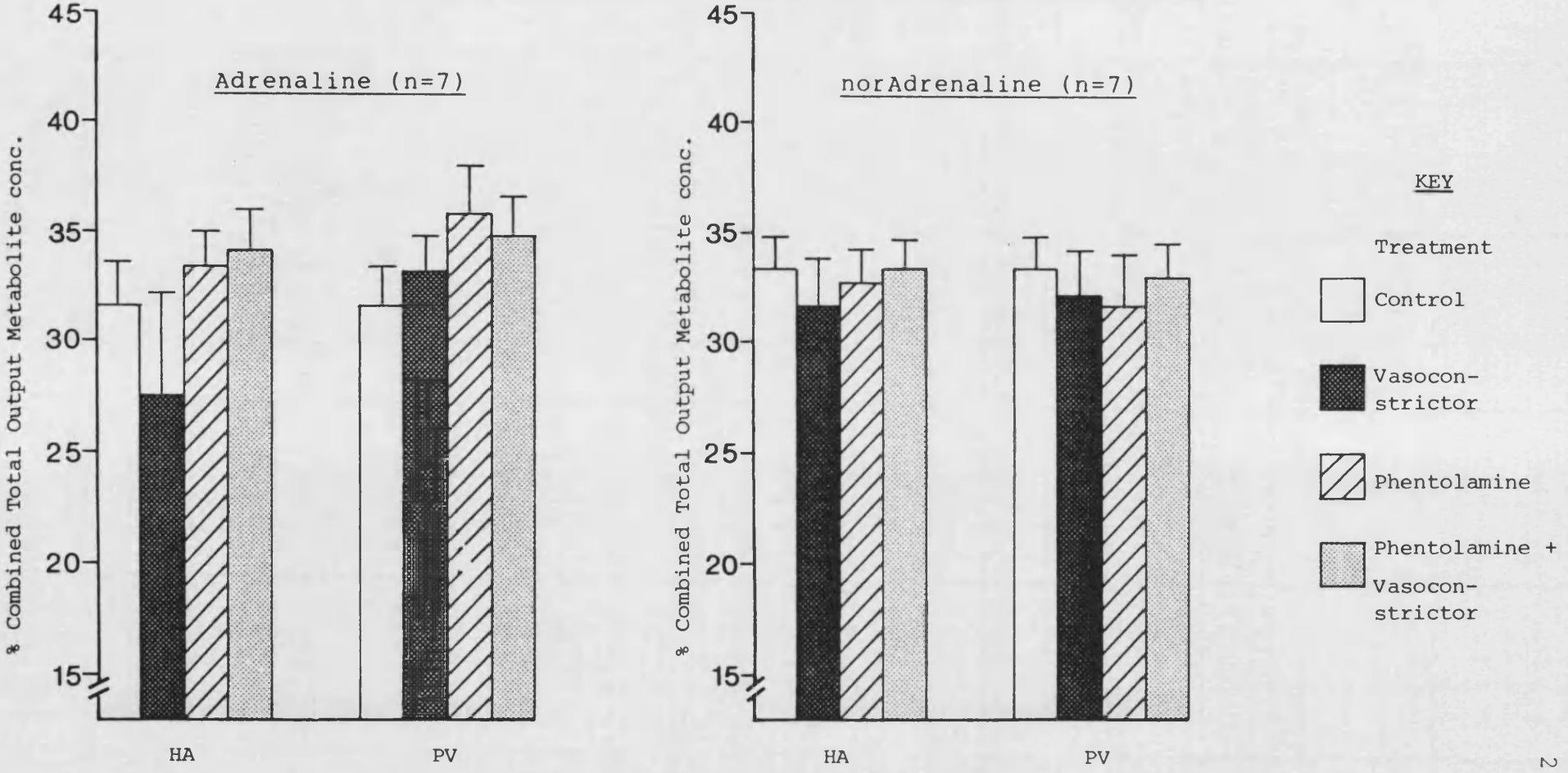
Bars are means + sem. Data is given in Appendix 30.

Figure 3.3.4. The Effect of Adrenaline and norAdrenaline Induced Increases in Perfusion Pressure on the Recovery of Total 3-OH MEGX. Values are Expressed as Percentages of Combined Total Output Metabolite Concentration.



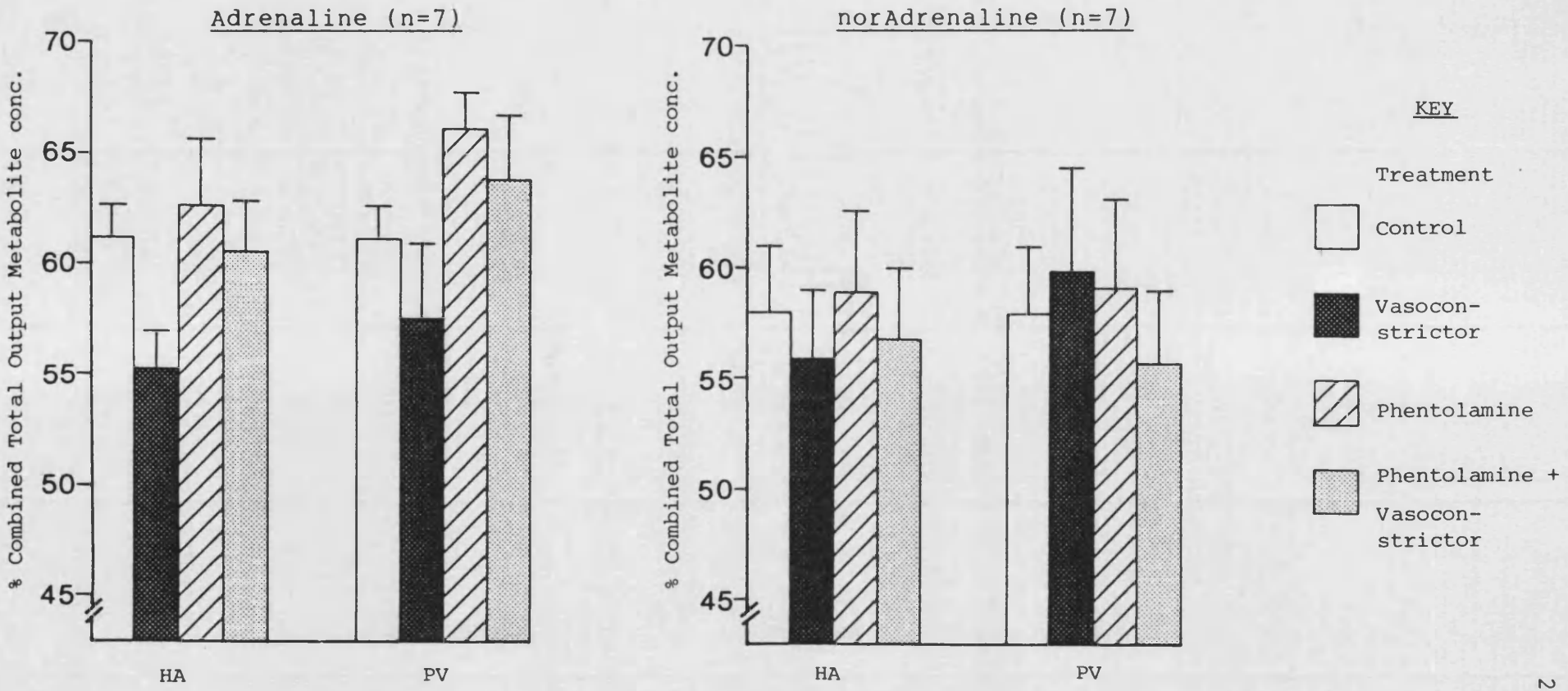
Bars are means + sem. Data is given in Appendix 31.

Figure 3.3.5. The Effect of Adrenaline and norAdrenaline Induced Increases in Perfusion Pressure on the Recovery of Total De-ethylated, (MEGX + GX) Metabolites. Values are Expressed as Percentages of Combined Total Output Metabolite Concentration.



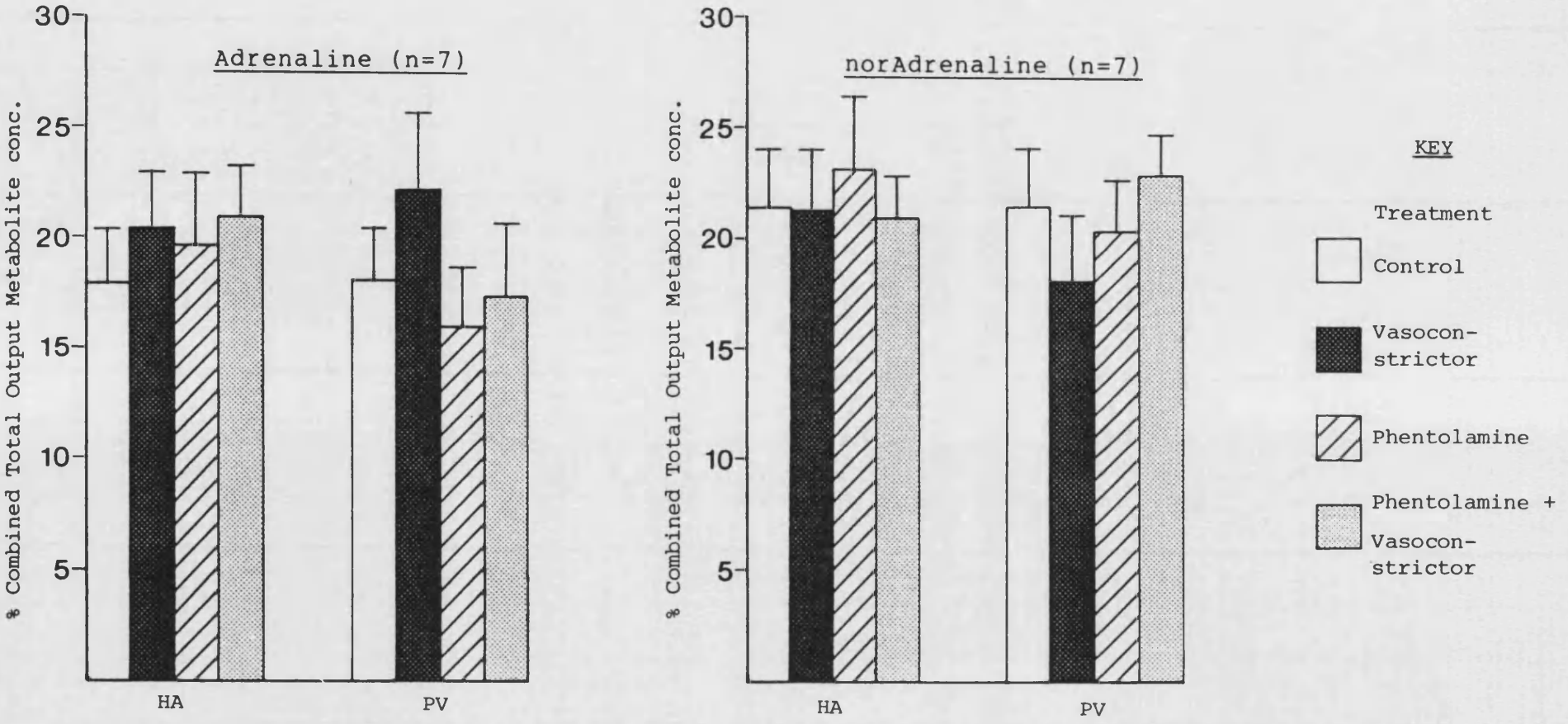
Bars are means + sem. Data is given in Appendix 32.

Figure 3.3.6. The Effect of Adrenaline and norAdrenaline Induced Increases in Perfusion Pressure on the Recovery of Total Glucuronides. Values are Expressed as Percentages of Combined Total Output Metabolite Concentration.



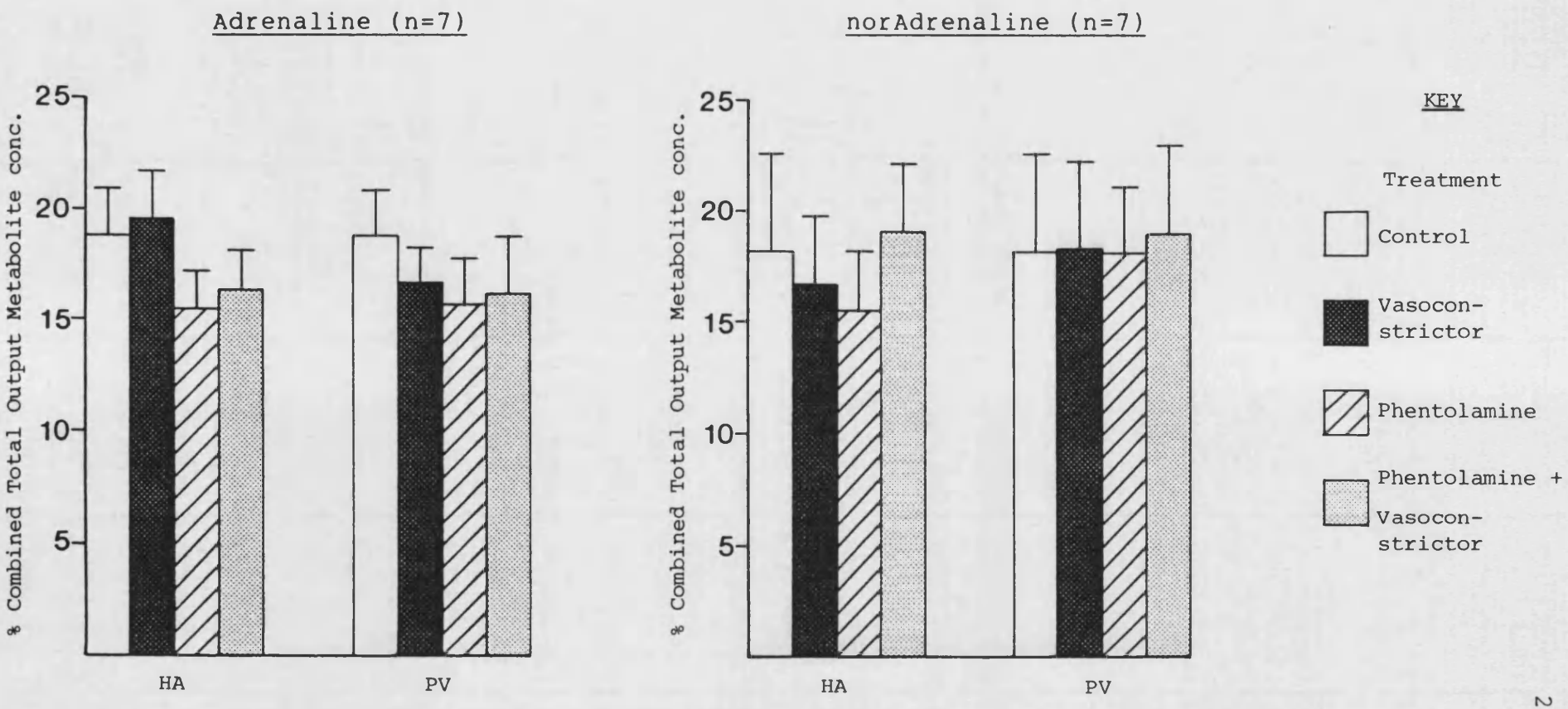
Bars are means + sem. Data is given in Appendix 33.

Figure 3.3.7. The Effect of Adrenaline and norAdrenaline Induced Increases in Perfusion Pressure on the Recovery of Total Sulphates. Values are Expressed as Percentages of Combined Total Output Metabolite Concentration.



Bars are means + sem. Data is given in Appendix 34.

Figure 3.3.8. The Effect of Adrenaline and norAdrenaline Induced Increases in Perfusion Pressure on the Recovery of Total Unconjugated Metabolites. Values are Expressed as Percentages of Combined Total Output Metabolite Concentration.



Bars are means + sem. Data is given in Appendix 35.

4) THE EFFECT OF HYDRALAZINE ON THE METABOLISM OF LIGNOCAINE.

Hydralazine has been reported to inhibit heart oxygenase systems but not the monoamine oxygenase systems in the rat liver, (Lyles et al., 1983). McLean et al. (1980) and Schneck et al. (1984) reported an increase in bioavailability of propranolol after hydralazine treatment. Heinzow et al. (1984) showed that hydralazine increased estimated liver blood flow in the dog but increased the bioavailability of propranolol. The results of Heinzow et al. (1984) would seem to be contradictory. Propranolol is a drug which is highly metabolised by the liver and its clearance is dependent on liver blood flow, (Wood et al., 1979 and Branch et al., 1973). An increase in liver blood flow would be expected to reduce bioavailability. Experiments by Johnston (1975), in rabbits showed that hydralazine increased heart rate, cardiac output and reduced total peripheral resistance, but total liver blood flow remained constant. Further investigations of the role of hydralazine in the alteration of the pharmacokinetics of co-administered drugs are required.

(a) Effect of hydralazine on the Extraction ratio of Lignocaine.

Hydralazine may exert its effect on drug kinetics by inhibition of their removal by the liver. To investigate this possibility lignocaine metabolism was studied in the isolated perfused rat liver during infusions of hydralazine. Rats were perfused at an HA:PV flow ratio of 2.5:7.5 ml/min. The method used for perfusion is described in chapter 2 sections 4,5 and 6. Hydralazine was added directly to the perfusate in each case. The results of table 3.4.1 show that infusions of hydralazine into the perfused liver had no effect on the extraction ratio of lignocaine. This indicates that hydralazine does not have a direct effect on the rate of removal of lignocaine from the circulation by the liver. Such alterations may however, be important for drugs other than lignocaine.

(b) Effect of hydralazine on the systolic blood pressure of the rat

Figure 3.4.1 shows the effect of hydralazine on the systolic blood pressure in the rat, (see Chapter 2 section 8e for methods). Between 30 and

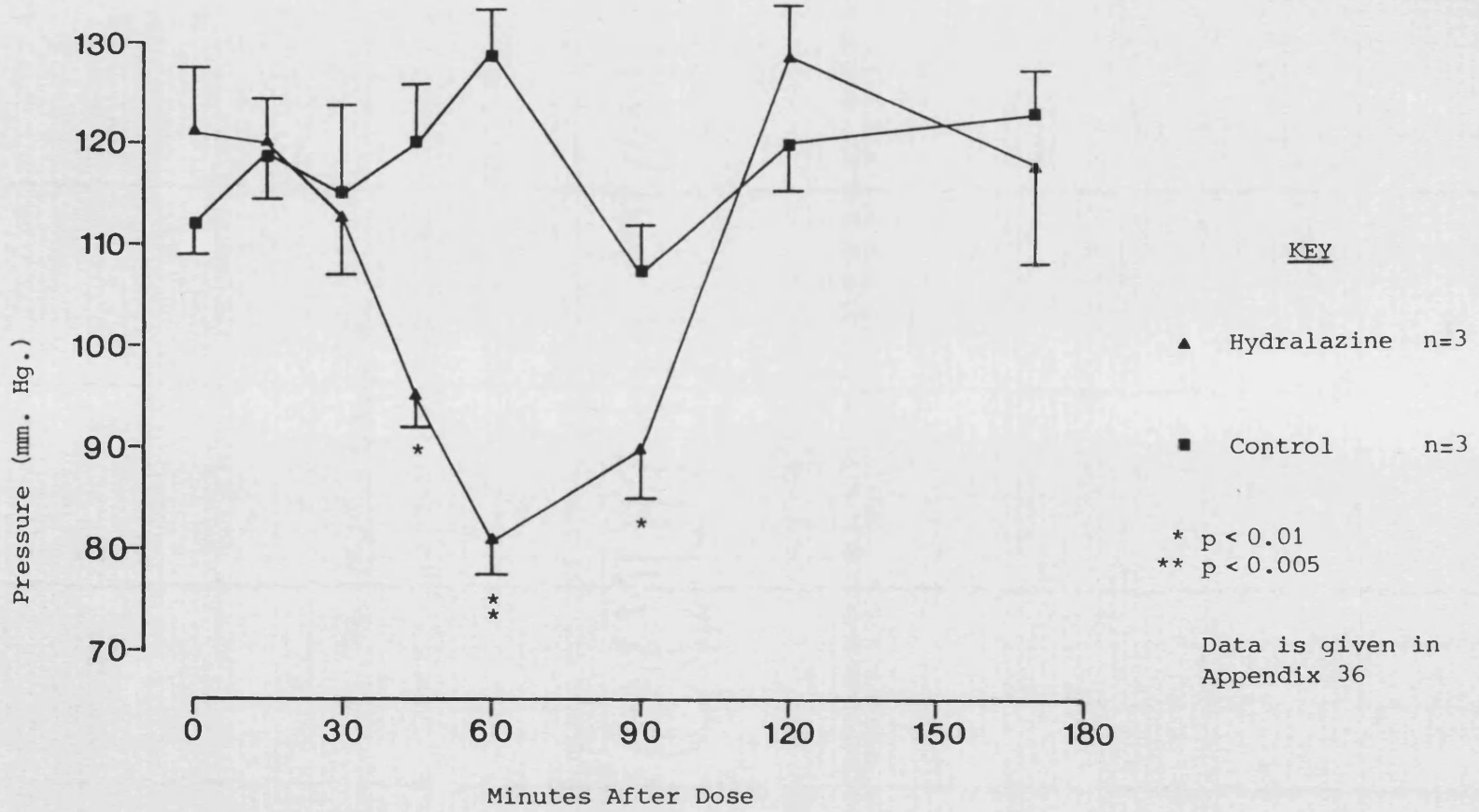
Table 3.4.1

To Show the Effect of Hydralazine on the Extraction Ratio of Lignocaine in the Perfused Rat Liver

Hydralazine Infusion concentration	Lignocaine Extraction Ratio (n=6) (mean ± s.e.m.)
0	97.96 ± 0.13
1×10^{-5} M	97.98 ± 0.09
5×10^{-5} M	97.85 ± 0.29
1×10^{-4} M	97.79 ± 0.27

Using the Mann Whitney test no significant difference was found between the extraction ratios of Lignocaine with and without various doses of Hydralazine.

Figure 3.4.1. The Effect of Hydralazine, 1mg/kg given Orally, on Systolic Blood Pressure in the Rat.



Points are Means ± sem.

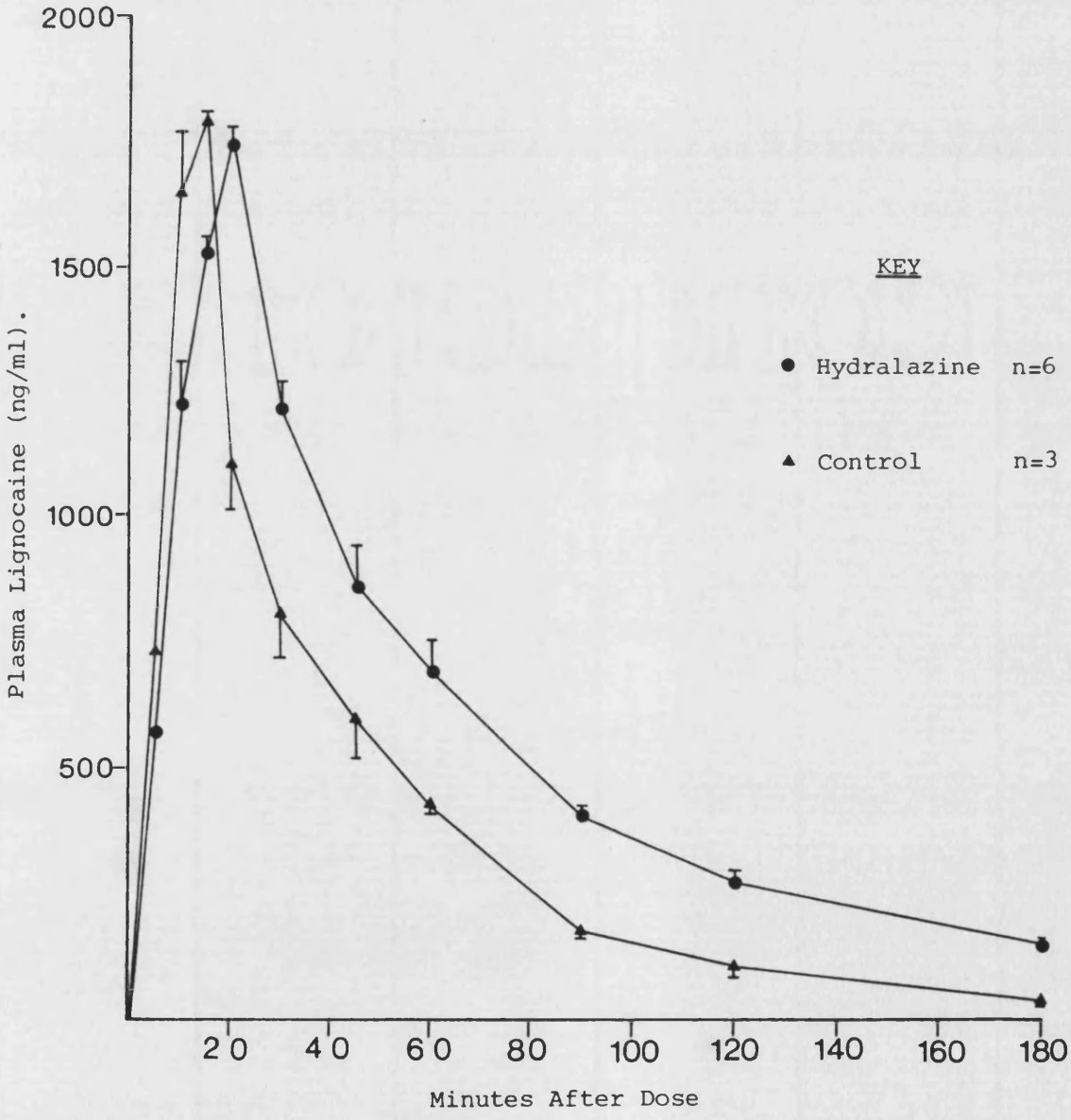
60 minutes after hydralazine (1mg/kg), given orally, the blood pressure falls. The blood pressure remains below control for a further forty minutes. Thus hydralazine produces a marked drop in blood pressure between 40 and 90 minutes after an oral dose.

(c) Effect of Hydralazine on the plasma Clearance of lignocaine

Figure 3.4.2 shows the effect of hydralazine on the plasma clearance of lignocaine, 20 mg/kg, given orally, (see chapter 2 section 8f for methods). Treatment with hydralazine, 1mg/kg, altered the plasma concentration/time curve for lignocaine. The pharmacokinetic parameters for the curves are given in table 3.4.2. Hydralazine treatment causes an increase in half life of the α phase, total elimination time and area under the curve. Figure 3.4.3 shows the effect of hydralazine treatment on lignocaine, 5mg/kg, given by an intravenous bolus injection. Hydralazine changes the plasma concentration curve of lignocaine. Table 3.4.2 presents the pharmocokinetic parameters for both control and treated curves. The results show that hydralazine treatment increases the α phase half life, total elimination time and area under the curve for lignocaine plasma elimination. The results indic-

Figure 3.4.2.

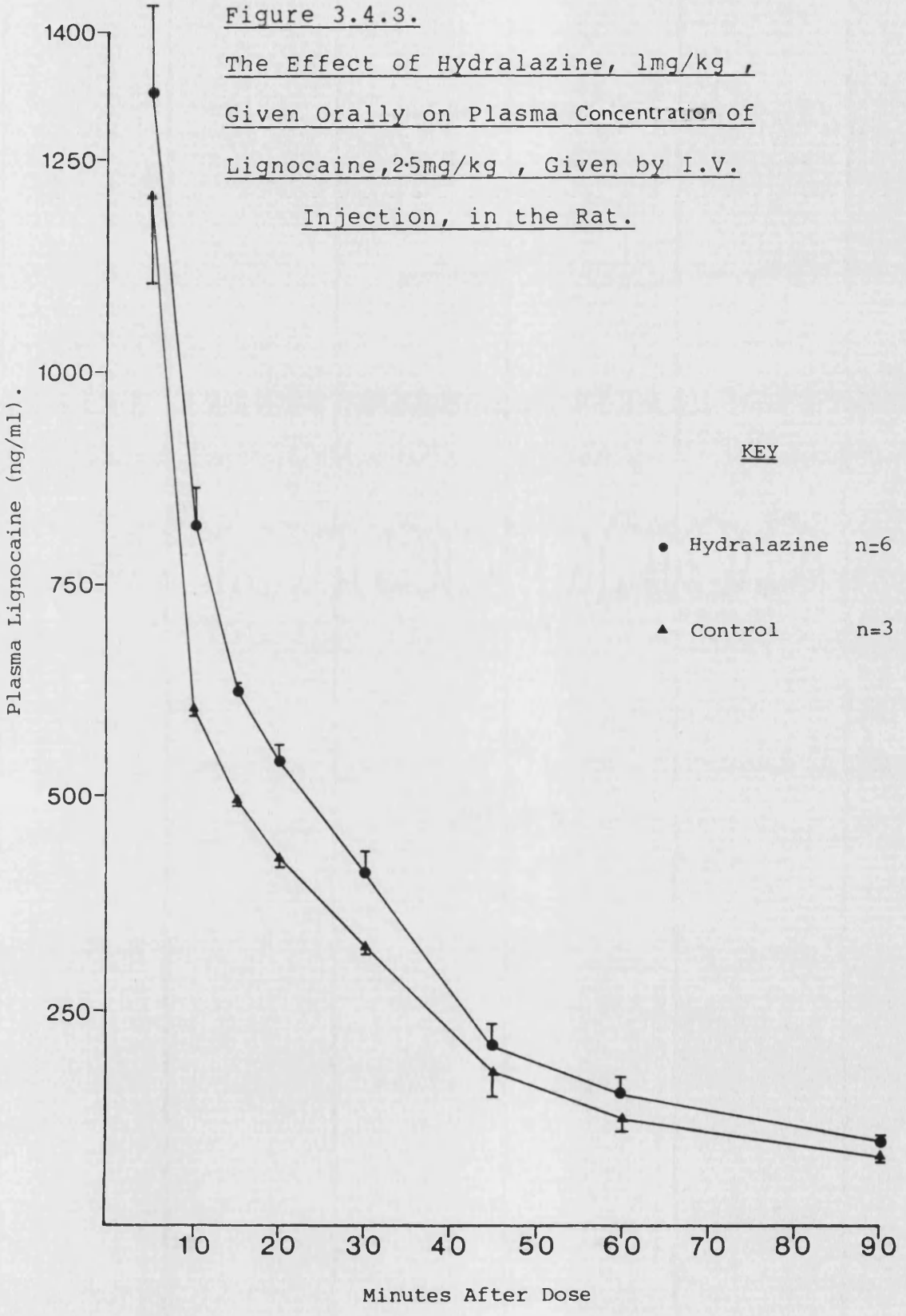
The Effect of Hydralazine, 1mg/kg , Given Orally on the Plasma Concentration of Lignocaine, 20mg/kg , Given Orally, in the Rat.



Points are means ± sem.
Data is given in Appendix 37

Figure 3.4.3.

The Effect of Hydralazine, 1mg/kg , Given Orally on Plasma Concentration of Lignocaine, 2·5mg/kg , Given by I.V. Injection, in the Rat.



Points are means ± sem.
Data is given in Appendix 37

Table 3.4.2

Pharmacokinetic Parameters of Single Oral and I.V. Doses of Lignocaine with and without Hydralazine Administration

Parameter	ORAL Lignocaine		I.V. Lignocaine	
	Control n=3	Hydralazine Treated n=6	Control n=3	Hydralazine Treated n=6
conc. at time 0 (ng/ml.)	-	-	1752	1866
Half life α Phase (mins)	27	40	7.7	9.2
Total Elimination Time (mins)	371	601	252	271
AUC 0-∞ (ng/ml.min)	73110	144462	35683	45093

$$\left(\frac{\text{Control Oral AUC}}{\text{Control I.V. AUC}}\right) = 2.05 \qquad \left(\frac{\text{Hydralazine Oral AUC}}{\text{Hydralazine I.V. AUC}}\right) = 3.20$$

Concentration at time zero was calculated using linear log concentration plots for the α phase.

Elimination time was calculated using linear log concentration plots for the β phase.

AUC 0-∞ was calculated using the the trapezoidal rule.

ate that hydralazine affects the clearance of lignocaine in the rat. Lignocaine has a high intrinsic clearance and extraction ratio (Branch et al., 1973; Shand et al., 1975; Ahmad et al., 1983 and Lennard et al., 1983), and as such its elimination can be considered to be blood flow dependent, (Nies et al., 1976). From section 4 (a) it was shown that lignocaine does not inhibit the enzymes responsible for lignocaine metabolism. Hydralazine must therefore be acting by another mechanism to affect the plasma elimination of lignocaine. Since the elimination of the drug is blood flow dependent hydralazine may decrease liver blood flow and reduce the rate of elimination.

(d) Effect of Hydralazine on liver blood flow

Figure 3.4.4 shows the effect of hydralazine on liver blood flow as measured using the dye indocyanine green, (see Chapter 2 section 8g for methods). The dye is extracted exclusively by the liver and does not undergo enterohepatic circulation, (Caesar et al., 1961). The removal of the dye is dependent on the blood flow to the liver and may be used as an estimate of relative liver blood flow. Table 3.4.3 shows the pharmacokinetic parameters

Figure 3.4.4. The Effect of Hydralazine, 1mg/kg , Given Orally on the Plasma Concentration of Indocyanine Green 6uM/kg , Given I.V., in the Rat.

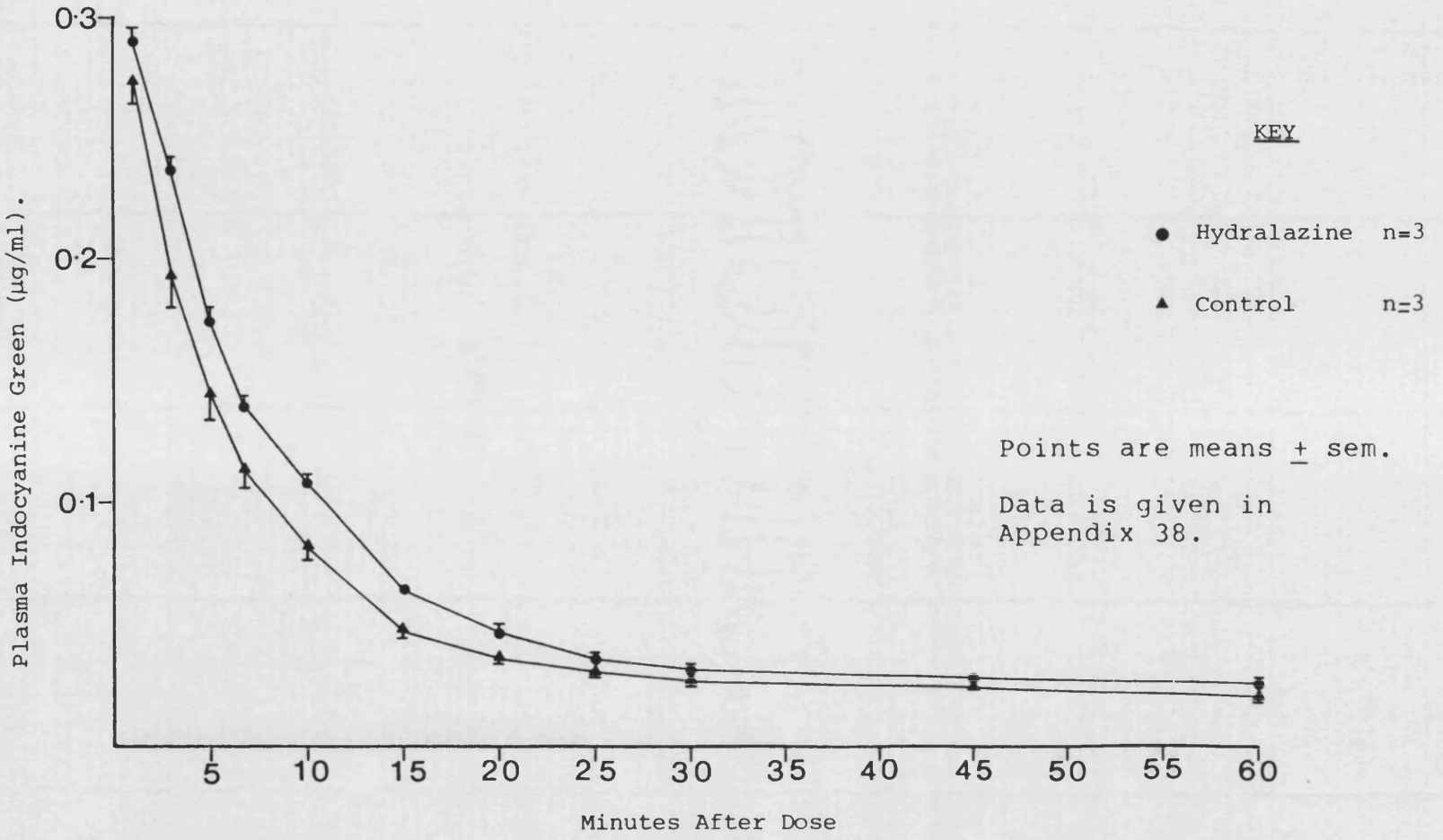


Table 3.4.3

Pharmacokinetic Parameters for the Plasma Indocyanine Clearance curves

PARAMETER	TREATMENT	
	Control	Hydralazine
Concentration at time 0	0.318 um/ml	0.336 um/ml
Total plasma Elimination Time	235 min	244 min
Half Life	4.33 min	** 5.38 min
Area Under the Curve 0 to ∞	4954.5 $ng\ ml^{-1}\ min$	6501.5 $ng\ ml^{-1}\ min$

Concentration at time zero was calculated linear log concentration plots for the α phase.

Total elimination time was calculated using linear log concentration plots for the β phase.

Area under the curve was calculated using the trapezoid rule.

** $p < 0.01$ using the unrelated "t" test.

for indocyanine green. The half life of the α phase may be used to estimate changes in liver blood flow. After treatment with Hydralazine, lmg/kg, orally, the half life of indocyanine green is significantly increased ($p<0.01$) from 4.33 to 5.38 minutes. This represents a 24% decrease in liver blood flow. From table 3.4.2 the same dose of hydralazine caused an increase in the half life of lignocaine, 2.5 mg/kg, when given by I.V. blous injection, from 7.7 to 9.2 minutes. This represents a 19.5% decrease in the rate of lignocaine elimination. Also from table 3.4.2 hydralazine treatment, (lmg/kg given orally) results in a 48% decrease in oral lignocaine elimination. Thus, the decrease in blood flow due to hydralazine can account for the reduction in removal of an IV dose of lignocaine but is insufficient to account for the decrease in elimination of lignocaine given orally. Hydralazine may also effect the rate at which an oral dose is absorbed in to the blood stream.

(e) Effect of hydralazine on lignocaine tissue binding

Post et al. (1979), demonstrated that lung tissue in rat may accumulate a considerable amount of lignocaine. Johnston (1975), reported that hydral-

azine increased kidney blood flow, however, he did not measure pulmonary blood flow. Hydralazine may increase pulmonary blood flow and cause increased accumulation of lignocaine to lung tissue. This accumulated drug will be eliminated from the body at a slower rate and will result in a reduction in the rate of plasma elimination of lignocaine. Table 3.4.4 shows the results of experiments to determine the binding of lignocaine to various tissues, (see Chapter 2 section 8h for method). The binding of lignocaine is determined using ^{14}C drug. The tissues are removed 2 minutes after an IV dose of lignocaine. This time period allows the drug to circulate within the body and also keeps the levels of circulating radioactive metabolites to a minimum. The results of the binding studies given in table 3.4.4 show that hydralazine, 1mg/kg, given orally did not significantly affect lignocaine binding to liver, kidney, heart or lung tissues.

(f) Effect of hydralazine on the binding of lignocaine to plasma proteins

Experiments by Piafsky et al. (1979), Routledge et al. (1980) and McNamara et al. (1981), have shown that lignocaine is between 50 and 70% bound to plasma protein especially albumin and α_1-

Table 3.4.4

The Effect of 1mg/kg Hydralazine given Orally on the Binding of Lignocaine to Various Tissues

	DRUG BOUND TO TISSUE ng/g of tissue			
	LIVER	KIDNEY	HEART	LUNG
CONTROL	3.88 ± 0.59	9.08 ± 0.78	2.94 ± 1.01	4.14 ± 0.63
HYDRALAZINE TREATED	2.60 ± 0.49	8.27 ± 1.16	1.81 ± 0.12	4.22 ± 0.99

Values are means ± standard error of the mean.

n=5 All values did not show a significant difference from controls using the Mann Whitney Test.

acid glycoprotein. Hydralazine could increase the binding of lignocaine to such plasma proteins and reduce the rate of lignocaine elimination. Table 3.4.5 shows the effect of incubating blood with 1×10^{-4} M hydralazine on the binding of lignocaine to plasma proteins and red blood cells, (see Chapter 2 section 8i for method). Hydralazine reduces the lignocaine bound to red blood cells and increases that bound to plasma proteins but has little effect on the free lignocaine. The changes are not sufficient to account for the reduction in lignocaine elmination observed with hydralazine treatment.

(g) The Effect of Hydralazine on the Isolated perfused heart.

Table 3.4.6 shows the effect of hydralazine on the heart rate in the isolated perfused heart, (see Chapter 2 section 8j for method). The normal heart rate in the isolated perfused heart preparations used varied between 224 and 248 beats/min with an average of 234 beats/min. The reductions in heart rate observed with 1×10^{-4} M and 1×10^{-3} M hydralazine are quite marked. The results are similar to those of Koch-Wesser (1974) who only showed a decrease in guinea-pig pacemaker activity at hydralazine concentrations above 10^{-4} M.

Table 3.4.5

To Show the Effect of 10^{-4} M Hydralazine on the Binding of Lignocaine to Plasma Proteins and Red Blood Cells

	Lignocaine conc. ug/ml.	DISTRIBUTION OF LIGNOCAINE IN BLOOD %		
		Protein Bound	Blood Cell Bound	Unbound
CONTROL	4.0	36.1	33.9	29.9
	1.0	39.3	33.8	26.8
	0.4	44.7	35.0	20.3
	0.1	46.6	32.8	20.6
HYDRALAZINE	4.0	51.9	29.7	18.4
	1.0	54.4	24.9	20.6
	0.4	56.0	28.7	15.3
	0.1	50.7	15.3	24.9

Pooled blood was obtained from 10 rats.

Table 3.4.6. The effect of Hydralazine on the Heart Rate in the Isolated Perfused Rat Heart

Hydralazine Treatment	Reduction in Heart Rate Beats/min			
	Mean	±	s.e.m.	n
1 X 10^{-4} M	30	±	2.8	3
1 X 10^{-3} M	83	±	6.5	3

Experiments by Ablad et al. (1962 and 1963) have shown that hydralazine may induce vasodilation in peripheral vessels in man. Hydralazine may thus reduce liver blood flow by a combination of a reduction in cardiac output due, to a decrease in heart rate and peripheral vasodilation. Both these factors may account for the decrease in blood pressure seen with hydralazine. However, the doses of hydralazine required to produce a decrease in heart rate are far in excess of the levels needed to reduce the rate of lignocaine elimination or decrease rat blood pressure. Hydralazine has been shown to be rapidly cleared from the systemic

circulation, (Ludden et al., 1980 and Reece et al., 1980), but its effect is much longer lasting due to formation of an active metabolite hydralazine acetone hydrazone. This metabolite has been shown to be more active than the parent compound at dilating rabbit aorta smooth muscle, Barron et al. (1977) and McLean et al. (1978). The action of these metabolites may be more important in the mechanism of action of hydralazine.

h) Summary of the investigtions on the interactions between lignocaine and hydralazine

The findings of the work on hydralazine can be summerised as follows.

i) The drug does not directly inhibit the metabolism of lignocaine.

ii) Hydralazine will reduce the rate of elimination of lignocaine from plasma.

iii) Hydralazine will reduce liver blood flow as measured by indocyanine green and this may account in part, for the reduction in lignocaine elimination.

iv) Binding of lignocaine to either plasma proteins, red blood cells and tissues is not altered by hydralazine.

v) The drug will produce a decrease in systolic blood pressure and at high concentrations a decrease in heart rate. The high concentrations needed to produce a reduction in heart rate discount this as the mechanism responsible for the fall in liver blood flow. However, metabolites of hydralazine may be more active and produce a reduction in both cardiac output and a peripheral vasodilation which could account for changes in liver blood flow and which could be the mechanism by which hydralazine reduces lignocaine elimination.

CHAPTER 4

CONCLUSIONS

In the isolated perfused rat liver, changes in the hepatic artery : portal vein (HA:PV) flow ratio result in an alterations in the extraction ratio of lignocaine. Increases in perfusion pressure in the hepatic artery are seen to reduce the extraction ratio of lignocaine to a similar degree to that seen during increases in hepatic artery flow contribution. The effect of the HA:PV flow ratio on lignocaine metabolism in the perfused liver seems to be a mechanical effect brought about by changes in pressure. An increase in arterio-venous shunting would seem to be the most likely explanation for the results, although changes in fenestral size are another possible explanation. Further investigations are required to determine the actual cause of these observed changes. Shunting may be easily measured using radiolabelled microspheres. The effect of increases in perfusion pressure on shunting could thus be determined. Measurement of fenestral diameter is much more difficult and requires the use of electron microscopy but using these techniques the effect of increases in perfusion pressure could be investigated.

Changes in the HA:PV flow ratio also resulted in a change in the metabolic profile of

lignocaine. As portal vein flow contribution was raised the recovery of de-ethylated metabolites increased and the recovery of hydroxylated metabolites decreased. Perfusion pressure changes were not responsible for the changes in the metabolic profile seen during changes in HA:PV flow ratio. The most likely explanation is that separate channels exist in the liver for the passage of hepatic artery and portal vein blood. These separate channels also possess heterogeneous metabolic environments. An increase in flow through one route exposes the blood to a more arterial or portal environment and this results in changes in metabolic profile. Heterogeneity between the hepatic artery and the portal vein could be of importance in drug metabolism and predictive kinetics. The route by which a drug enters the liver may, in part, determine its metabolism. A drug may produce more toxic metabolites during passage through the arterial route than by the portal route. Subjects who have an altered blood flow ratio either due to a surgical shunt, liver damage or as a result of drug induced increases in flow may treat a therapeutic agent differently from a normal subject.

The existence of separate channels is still a matter of controversy. The results of this

study support their existence but their importance in drug metabolism and liver function has yet to be determined. The changes in flow induced in this study are large and would only be expected to occur in the body under extreme circumstances. Studies of flow ratio relationships for other drugs are required to determine whether a general pattern of heterogeneity exists. Also studies of the distribution of cytochromes P_{450} and conjugating enzymes between the two routes are required.

The study also shows that although both the hepatic artery and the portal vein possess α-adrenoreceptors and β-adrenoreceptors, heterogeneity exists in the distribution of β-adrenoreceptor subtypes. The hepatic artery having a greater β_1-adrenoreceptor population and the portal vein a greater β_2-adrenoreceptor population. The reason for this heterogeneity is uncertain. If a similar heterogeneity occurs in man it may provide a possible selective treatment for portal hypertension with minimum effects on general circulation.

Care must be taken when using data from isolated preparation even in the same species. In this study the interaction between hydralazine and lignocaine were investigated. Hydralazine was shown to

reduce the plasma clearance of lignocaine in the whole rat but was ineffective on hepatic enzymes. Hydralazine reduced liver blood flow in the whole animal and it was found that the most likely cause of its effect on lignocaine clearance was due to its effects on cardiac output. Thus, changes in liver vasculature resistance in isolated preparation due to infusions of selective β_1 and β_2-adrenoreceptor agonists and antagonists, may be secondary to their effects on the general circulation.

CHAPTER 5

APPENDICES

Appendix 1

To Show the concentrations in Salts used in the Krebs Buffer for Liver Perfusion Experiments

Substance	Final concentration m mole/litre
Sodium Chloride	118.4
Glucose	11.1
Sodium Bicarbonate	25.0
Potassium Chloride	4.7
Magnesium Sulphate Heptohydrate	1.1
Calcium Chloride	2.5
Potassium Dihydrogen orthophosphate	1.2

Appendix 2

To Show the concentrations in Salts used in the Krebs Buffer for Heart Perfusion Experiments

Substance	Final concentration m mole/litre
Sodium Chloride	118.4
Glucose	11.0
Sodium Bicarbonate	25.0
Potassium Chloride	4.7
Magnesium Sulphate Heptohydrate	1.2
Calcium Chloride	1.2
Potassium Dihydrogen orthophosphate	1.2

Appendix 3

The Effect of Changes in the PV:HA Flow Ratio on the Extraction Ratio of Lignocaine

FLOW RATIO HA : PV	Lignocaine Extraction Ratio	Standard Error	n Value
7.5 : 2.5	93.18	± 0.40	20
5.0 : 5.0	96.07	± 0.18	40
2.5 : 7.5	97.83	± 0.09	75
1.0 : 9.0	98.78	± 0.19	15
0 : 10.0	99.20	± 0.05	70

$$ER = \left(\frac{C_{in} - C_{out}}{C_{in}} \right) X\ 100$$

ER = Extraction ratio

C_{in} = Input Lignocaine oncentration in both the Hepatic Artery and the Portal Vein.

C_{out} = Output Lignocaine concentration

Appendix 4. The Effect of Variations in the HA:PV Flow Ratio On the Recovery 3-OH Lignocaine and its Conjugates. Values are Expressed as a Percentage of Combined Total Output Metabolite Concentration in nM/ml.

FLOW (ml/min) HA : PV	Total 3-OH Lig	3-OH Lig Glucuronide	3-OH Lig Sulphate	Unconjugated 3-OH Lig
5 : 5 n = 37 (8 rats)	28.92 ± 0.79 ***	8.32 ± 1.11 ***	16.00 ± 1.41 ***	4.62 ± 0.17 ***
2.5 : 7.5 n = 35 (7 rats)	22.93 ± 1.03	16.73 ± 0.92	2.98 ± 0.50	3.16 ± 0.26
0 : 10.0 n = 48 (10 rats)	17.39 ± 1.08 ***	9.72 ± 1.00 ***	4.05 ± 0.42 *	4.07 ± 0.30

Values are Percentages ± Standard errors of the Mean.

Significance was Measured from the 2.5 : 7.5 Flow Ratio by the Unrelated "t" test.

Significance

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Appendix 5. The Effect of Variations in the HA:PV Flow Ratio On the Recovery 3-OH MEGX and its Conjugates. Values are Expressed as a Percentage of Combined Total Output Metabolite Concentration in nM/ml.

FLOW (ml/min) HA : PV	Total 3-OH MEGX	3-OH MEGX Glucuronide	3-OH MEGX Sulphate	Unconjugated 3-OH MEGX
5 : 5 n = 37 (8 rats)	43.51 ± 0.99	31.34 ± 0.95	6.87 ± 0.61	5.31 ± 0.45 ***
7.5 : 2.5 n = 35 (7 rats)	45.34 ± 0.87	25.61 ± 1.80	8.79 ± 0.84	10.94 ± 0.93
0 :10 n = 48 (10 rats)	47.48 ± 0.90	28.00 ± 1.14	13.43 ± 1.24 **	5.87 ± 0.46 ***

Values are Percentages ± Standard errors of the mean.

Significance was Measured from the 2.5 : 7.5 Flow Ratio by the Unrelated "t" test.

Significance

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Appendix 6. The Effect of Variations in the HA:PV Flow Ratio On the Recovery GX and its Conjugates. Values are Expressed as a Percentage of Combined Total Output Metabolite Concentration in nM/ml.

FLOW (ml/min) HA : PV	Total GX	GX Glucuronide	GX Sulphate	Unconjugated GX
5 : 5 n = 37 (8 rats)	21.98 ± 0.59	4.15 ± 0.52 ***	2.50 ± 0.39	15.37 ± 0.79 ***
2.5 : 7.5 n = 35 (7 rats)	21.36 ± 0.75	10.63 ± 0.93	4.06 ± 0.56	6.65 ± 0.70
0 : 10 n = 48 (10 rats)	24.06 ± 0.82	8.13 ± 0.81	8.91 ± 1.02 ***	7.03 ± 0.47

Values are Percentages ± Standard errors of the mean.

Significance was Measured from the 2.5 : 7.5 Flow Ratio by the Unrelated "t" test.

Significance

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Appendix 7. The Effect of Variations in the HA:PV Flow Ratio On the Recovery MEGX and its Conjugates. Values are Expressed as a Percentage of Combined Total Output Metabolite Concentration in nM/ml.

FLOW (ml/min) HA : PV	Total MEGX	MEGX Glucuronide	MEGX Sulphate	Unconjugated MEGX
5 : 5 n = 37 (8 rats)	0 ± 0 ***	0 ± 0 ***	0 ± 0 ***	0 ± 0 ***
2.5 : 7.5 n = 35 (7 rats)	6.83 ± 0.57	3.05 ± 0.35	3.19 ± 0.50	0.56 ± 0.11
0 : 10 n = 48 (10 rats)	8.70 ± 0.34 **	2.69 ± 0.32	4.22 ± 0.33	1.78 ± 0.32 **

Values are Percentages ± Standard errors of the mean.

Significance was Measured from the 2.5 : 7.5 Flow Ratio by the Unrelated "t" test.

Significance

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Appendix 8. The Effect of Variations in the HA:P V Flow Ratio On the Recovery of De-ethythylated, (GX + MEGX) Metabolites and Their Conjugates. Values are Expressed as a Percentage of Combined Total Output Metabolite Concentration in nM/ml.

FLOW (ml/min) HA : PV	Combined Total	Deethylated Glucuronides	Deethylated Sulphates	Unconjugated
5 : 5 n = 37 (8 rats)	21.98 ± 0.59 ***	4.15 ± 0.52 ***	2.50 ± 0.39 ***	15.37 ± 0.79 ***
2.5 : 7.5 n = 35 (7 rats)	28.19 ± 0.91	13.17 ± 0.89	7.26 ± 0.63	7.22 ± 0.69
0 : 10 n = 48 (10 rats)	32.51 ± 0.83 **	11.03 ± 0.87	13.29 ± 1.21 **	8.83 ± 0.55

Values are Percentages ± Standard errors of the mean.

Significance was Measured from the 2.5 : 7.5 Flow Ratio by the Unrelated "t" Test.

Significance

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Appendix 9. The Effect of Variations in the HA:PV Flow Ratio On the Recovery of Total Glucuronudes, Sulphates and Unconjugated Metabolies. Values are Expressed as a Percentage of Combined Total Output Metabolite Concentration in nM/ml.

FLOW (ml/min) HA : PV	Total Glucuronides	Total Sulphates	Total Unconjugated
5 : 5 n = 37 (8 rats)	43.76 ± 1.87 ***	25.35 ± 1.32 ***	25.29 ± 0.84 ***
2.5 : 7.5 n = 35 (7 rats)	55.76 ± 1.48	19.04 ± 0.97	21.36 ± 0.91 ***
0 : 10 n = 48 (10 rats)	48.90 ± 1.21 ***	30.34 ± 1.54 ***	18.93 ± 0.87 ***

Values are Percentages ± Standard errors of the mean

Significance was Measured from the 2.5 : 7.5 Flow Ratio by the Unrelated "t" Test.

Significance

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Appendix 10. The Effect of Alterations in the HA:PV Flow Ratio on the Recovery of Total Phase 1 Metabolites During infusion of various Lignocaine Metabolites

Metabolite Infused	CHANGE IN % DOSE (n=6)				
	Combined Total	Total 3-OH LIG	Total 3-OH MEGX	Total MEGX	Total GX
3-OH LIG	- 1.6 ± 1.5	- 1.0 ± 1.4	+16.8 ± 1.6	-14.2 ± 0.9	nd
sig			**	**	
3-OH MEGX	-15.3 ± 4.8	nd	+ 9.7 ± 0.7	-24.2 ± 4.1	- 0.7 ± 0.6
sig	*		**	**	
MEGX	- 2.5 ± 3.5	nd	+ 6.3 ± 2.0	- 3.1 ± 2.0	- 5.6 ± 2.2
sig			*		
GX	nd	nd	nd	nd	-13.2 ± 1.0
sig					*

Values Show changes in Percentage Dose Recovered when HA:PV Flow ratio is altered from 5 : 5 to 0 : 10 ml/min. ± standard error of the mean.

A negative value indicates a decrease in metabolite production as portal vein flow contribution is increased.

"nd" indicates that the metabolite was not detected.

Significance was assessed using the Wilcoxon test.

* $p < 0.025$

** $p < 0.01$

All other changes were not statistically significant.

Appendix 11. The Effect of Alterations in the HA:PV Flow Ratio on the Recovery of Glucuronides During Infusion of various Lignocaine Metabolites

Metabolite Infused	CHANGE IN % DOSE (n=6)				
	Total Glucur-onides	3-OH LIG Glucur-onide	3-OH MEGX Glucur-onide	MEGX Glucur-onide	GX Glucur-onide
3-OH LIG	+ 9.2 ± 2.5	-4.07 ± 1.3	+ 7.8 ± 1.6	- 2.7 ± 0.9	nd
sig	*				
3-OH MEGX	+ 2.4 ± 4.3	nd	+ 1.6 ± 0.6	+ 0.8 ± 4.2	- 0.0 ± 0.2
sig					
MEGX	+ 3.7 ± 1.7	nd	+ 7.7 ± 0.9	- 3.0 ± 1.8	- 1.0 ± 0.8
sig	*		*		
GX	nd	nd	nd	nd	+ 1.7 ± 1.0
sig					

Values Show changes in Percentage Dose Recovered when HA:PV Flow ratio is altered from 5 : 5 to 0 : 10 ml/min. ± standard error of the mean.

A negative value indicates a decrease in metabolite production as portal vein flow contribution is increased.

"nd" indicates that the metabolite was not detected.

Significance was assessed using the Wilcoxon test.

* $p < 0.025$

** $p < 0.01$

All other changes were not statistically significant.

Appendix 12. The Effect of Alterations in the HA:PV Flow Ratio on the Recovery of Sulphate Conjugates During Infusions of various Lignocaine Metabolites

Metabolite Infused	CHANGE IN % DOSE (n=6)				
	Total Sulpha-tes	3-OH LIG Sulphate	3-OH MEGX Sulphate	MEGX Sulphate	GX Sulphate
3-OH LIG	- 3.5 ± 1.9	+ 2.8 ± 0.7	+ 5.2 ± 0.8	-11.4 ± 1.7	nd
sig			**	**	
3-OH MEGX	-26.8 ± 1.5	nd	+ 0.1 ± 0.7	-27.3 ± 0.8	+ 0.2 ± 0.5
sig	**			**	
MEGX	+ 9.9 ± 2.4	nd	+ 2.1 ± 1.8	+ 9.3 ± 1.5	- 1.8 ± 1.5
sig				**	
GX	nd	nd	nd	nd	+ 0.2 ± 0.6
sig					

Values Show changes in Percentage Dose Recovered when HA:PV Flow ratio is altered from 5 : 5 to 0 : 10 ml/min. ± standard error of the mean.

A negative value indicates a decrease in metabolite production as portal vein flow contribution is increased.

"nd" indicates that the metabolite was not detected.

Significance was assessed using the Wilcoxon test.

* $p < 0.025$

** $p < 0.01$

All other changes were not statistically significant.

Appendix 13. The Effect of Alterations in the HA:PV Flow Ratio on the Recovery of Unconjugated or Free Metabolites During Infusions of various Lignocaine Metabolites

Metabolite Infused	CHANGE IN % DOSE (n=6)				
	Total Free	Free 3-OH LIG	Free 3-OH MEGX	Free MEGX	Free GX
3-OH LIG	- 4.0 ± 1.4	- 7.9 ± 0.6	+ 3.9 ± 1.2	0 ± 0	nd
sig		**			
3-OH MEGX	+ 9.1 ± 0.8	nd	+ 8.2 ± 0.5	+ 2.2 ± 0.7	- 1.3 ± 0.5
sig	**		**	*	
MEGX	-12.4 ± 2.5	nd	- 0.5 ± 0.3	- 9.1 ± 1.8	- 2.8 ± 1.4
sig	**			**	
GX	nd	nd	nd	nd	-15.2 ± 1.4
sig					**

Values Show changes in Percentage Dose Recovered when HA:PV Flow ratio is altered from 5 : 5 to 0 : 10 ml/min. ± standard error of the mean.

A negative value indicates a decrease in metabolite production as portal vein flow contribution is increased.

"nd" indicates that the metabolite was not detected.

Significance was assessed using the Wilcoxon test.

* $p < 0.025$

** $p < 0.01$

All other changes were not statistically significant.

Appendix 14. The Effect of Phentolamine on the ED50% of Adrenaline and norAdrenaline in the Hepatic Artery

Phentolamine Dose	CHANGE IN ED50% OF CONSTRICTOR			
	sig	Adrenaline	sig	norAdrenaline
5×10^{-5} M	***	2.08 ± 0.07 n=3	***	2.05 ± 0.01 n=3
5×10^{-6} M	***	0.90 ± 0.04 n=3	***	1.35 ± 0.02 n=4
5×10^{-7} M	***	0.40 ± 0.05 n=3	***	0.60 ± 0.02 n=3

Values are mean changes in ED50% ± standard error of the mean.

Significance was measured by the related "t" test.

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Appendix 15. The Effect of Phentolamine on the ED50% of Adrenaline and norAdrenaline in the Portal Vein

Phentolamine Dose	CHANGE IN ED50% OF CONSTRICTOR			
	sig	Adrenaline	sig	norAdrenaline
1×10^{-5} M	**	1.65 ± 0.41 n=3	***	1.21 ± 0.10 n=5
1×10^{-6} M	*	0.46 ± 0.15 n=3	***	0.82 ± 0.07 n=3
5×10^{-7} M		0.32 ± 0.19 n=3	*	0.33 ± 0.12 n=3

Values are mean changes in ED50% ± standard error of the mean.

Significance was assessed using the related "t" test.

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Appendix 16. The Effect of Isoprenaline on the ED50% of Adrenaline and norAdrenaline in the Hepatic Artery

Isoprenaline Dose	CHANGE IN ED50% OF CONSTRICTOR			
	sig	Adrenaline	sig	norAdrenaline
5×10^{-5} M	***	0.80 ± 0.02 n=6	***	0.65 ± 0.03 n=6
5×10^{-6} M		0.03 ± 0.01 n=3		0.06 ± 0.03 n=3

Appendix 17. The Effect of Isoprenaline on the ED50% of Adrenaline and norAdrenaline in the Portal Vein

Isoprenaline Dose	CHANGE IN ED50% OF CONSTRICTOR			
	sig	Adrenaline	sig	norAdrenaline
1×10^{-5} M	***	1.00 ± 0.12 n=5	***	1.14 ± 0.09 n=5

Values for both tables are mean changes in ED50% $\pm$ standard error of the mean.

Significance for both tables was assessed by the related "t" test.

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Appendix 18. The Effect of Clenbuterol on the ED50% of Adrenaline and norAdrenaline in the Hepatic Artery

Clenbuterol Dose	CHANGE IN ED50% OF CONSTRICTOR			
	sig	Adrenaline	sig	norAdrenaline
5×10^{-5} M	***	0.28 ± 0.04 n=3	**	0.19 ± 0.04 n=3

Appendix 19. The Effect of Clenbuterol on the ED50% of Adrenaline and norAdrenaline in the Portal Vein

Clenbuterol Dose	CHANGE IN ED50% OF CONSTRICTOR			
	sig	Adrenaline	sig	norAdrenaline
1×10^{-5} M	***	0.87 ± 0.09 n=5	***	1.03 ± 0.08 n=5

Values for both tables are mean changes in ED50% ± standard error of the mean.

Significance for both tables was assessed by the related "t" test.

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Appendix 20. The Effect of Dobutamine on the ED50% of Adrenaline and norAdrenaline in the Hepatic Artery

Dobutamine Dose	CHANGE IN ED50% OF CONSTRICTOR			
	sig	Adrenaline	sig	norAdrenaline
5×10^{-6} M	***	2.35 ± 0.07 n=4	***	1.72 ± 0.03 n=4
1×10^{-6} M	***	0.57 ± 0.02 n=8	***	0.57 ± 0.12 n=8
5×10^{-7} M	***	0.24 ± 0.03 n=3		0.03 ± 0.02 n=3

Values are mean changes in ED50% ± standard error of the mean.

Significance was assessed using the related "t" test.

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Appendix 21. The Effect of Dobutamine on the ED50% of Adrenaline and norAdrenaline in the Portal Vein

Dobutamine Dose	CHANGE IN ED50% OF CONSTRICTOR			
	sig	Adrenaline	sig	norAdrenaline
1×10^{-5} M	***	0.66 ± 0.10 n=6	**	0.75 ± 0.17 n=6
1×10^{-6} M	***	0.11 ± 0.02 n=3	*	0.08 ± 0.02 n=3

Values are mean changes in ED50% ± standard error of the mean.

Significance was assessed using the related "t" test.

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

Appendix 22. The Effect of Various Beta Blockers on the shift in Adrenaline and norAdrenaline ED50% due to 5×10^{-5} M Isoprenaline in the Hepatic Artery

BLOCKER	CONCENTRATION	CONSTRICTOR INFUSED			
		Adrenaline n=3		norAdrenaline n=3	
		% Reduction	sig	% Reduction	sig
Atenolol	5×10^{-6} M	64 ± 12.0	***	99 ± 4.6	***
Propranolol	5×10^{-6} M	68 ± 7.8	***	80 ± 11.0	***
Metoprolol	5×10^{-6} M	44 ± 2.3	***	83 ± 3.1	***
Oxprenolol	5×10^{-6} M	70 ± 12.0	***	96 ± 1.2	***
ICI 118 551	5×10^{-6} M	4 ± 3.4		16 ± 6.2	

Values are mean % reduction in the Isoprenaline shift in ED50% of the vasoconstrictors Adrenaline and norAdrenaline ± standard error of the mean.

Significance was assessed using the related "t" test.

*** $p < 0.005$

All other values were not statistically significant.

Appendix 23. The Effect of Various Beta Blockers on the shift in Adrenaline and norAdrenaline ED50% due to 1×10^{-5} M Isoprenaline in the Portal Vein

BLOCKER	CONCENTRATION	CONSTRICTOR INFUSED			
		Adrenaline n=3		norAdrenaline n=3	
		% Reduction	sig	% Reduction	sig
Atenolol	1×10^{-5} M	32 ± 7.6	*	19 ± 9.0	
Propranolol	1×10^{-5} M	37 ± 8.2		38 ± 11.5	*
Metoprolol	1×10^{-5} M	22 ± 7.9		-	
Oxprenolol	1×10^{-5} M	-		-	
ICI 118 551	1×10^{-5} M	85 ± 5.8	***	80 ± 3.5	***

Values are mean % reduction in the Isoprenaline shift in ED50% of the vasoconstrictors Adrenaline and norAdrenaline ± standard error of the mean.

Significance was assessed using the related "t" test.

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

All other values were not statistically significant.

Appendix 24. The Effect of Various Beta Blockers on the shift in Adrenaline and norAdrenaline ED50% due to 5×10^{-5} M Clenbuterol in the Hepatic Artery

BLOCKER	CONCENTRATION	CONSTRICTOR INFUSED			
		Adrenaline n=3		norAdrenaline n=3	
		% Reduction	sig	% Reduction	sig
Atenolol	5×10^{-6} M	75 ± 3.8	***	77 ± 9.9	**
Propranolol	5×10^{-6} M	33 ± 10.3	**	54 ± 2.0	***
Metoprolol	5×10^{-6} M	55 ± 7.3	**	68 ± 11.9	**
Oxprenolol	5×10^{-6} M	75 ± 13.9	*	68 ± 6.0	**
ICI 118 551	5×10^{-6} M	31 ± 9.3		77 ± 14.0	*

Values are mean % reduction in the Clenbuterol shift in ED50% of the vasoconstrictors Adrenaline and norAdrenaline ± standard error of the mean.

Significance was assessed using the related "t" test.

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

All other values were not statistically significant.

Appendix 25. The Effect of Various Beta Blockers on the shift in Adrenaline and norAdrenaline ED50% due to 1×10^{-5} M Clenbuterol in the Portal Vein

BLOCKER	CONCENTRATION	CONSTRICTOR INFUSED			
		Adrenaline n=3		norAdrenaline n=3	
		% Reduction	sig	% Reduction	sig
Atenolol	1×10^{-5} M	10 ± 4.8		20 ± 8.3	
Propranolol	1×10^{-5} M	48 ± 10.0		14 ± 5.9	
Metoprolol	1×10^{-5} M	-5 ± 5.6		17 ± 5.4	*
Oxprenolol	1×10^{-5} M	-		-	
ICI 118 551	1×10^{-5} M	104 ± 17.0	**	108 ± 3.9	***

Values are mean % reduction in the Clenbuterol shift in ED50% of the vasoconstrictors Adrenaline and norAdrenaline ± standard error of the mean.

Significance was assessed using the related "t" test.

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

All other values were not statistically significant.

Appendix 26. The Effect of Various Beta Blockers on the shift in Adrenaline and norAdrenaline ED50% due to 1×10^{-6} M Dobutamine in the Hepatic Artery

BLOCKER	CONCENTRATION	CONSTRICTOR INFUSED			
		Adrenaline n=3		norAdrenaline n=3	
		% Reduction	sig	% Reduction	sig
Atenolol	5×10^{-6} M	52 ± 8.7	***	69 ± 13.0	**
Propranolol	5×10^{-6} M	106 ± 8.6	***	82 ± 6.7	***
Metoprolol	5×10^{-6} M	69 ± 5.7	***	100 ± 1.1	***
Oxprenolol	5×10^{-6} M	55 ± 3.9	***	79 ± 9.6	***
ICI 118 551	5×10^{-6} M	8 ± 3.4		19 ± 7.0	*

Values are mean % reduction in the Dobutamine shift in ED50% of the vasoconstrictors Adrenaline and norAdrenaline ± standard error of the mean.

Significance was assessed using the related "t" test.

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

All other values were not statistically significant.

Appendix 27. The Effect of Various Beta Blockers on the shift in Adrenaline and norAdrenaline ED50% due to 1×10^{-5} M Dobutamine in the Portal Vein

BLOCKER	CONCENTRATION	CONSTRICTOR INFUSED			
		Adrenaline n=3		norAdrenaline n=3	
		% Reduction	sig	% Reduction	sig
Atenolol	1×10^{-5} M	68 ± 9.3	***	43 ± 2.6	***
Propranolol	1×10^{-5} M	46 ± 13.8	*	38 ± 8.0	*
Metoprolol	1×10^{-5} M	30 ± 7.2	*	34 ± 7.1	
Oxprenolol	1×10^{-5} M	43 ± 12.0	*	50 ± 15.0	
ICI 118 551	1×10^{-5} M	20 ± 5.8		108 ± 3.9	*

Values are mean % reduction in the Dobutamine shift in ED50% of the vasoconstrictors Adrenaline and norAdrenaline ± standard error of the mean.

Significance was assessed using the related t test.

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$

All other values were not statistically significant.

Appendix 28

The Effect of Increases in Perfusion Pressure by Adrenaline or norAdrenaline Infusions On the Extraction Ratio of Lignocaine

TREATMENT	CONSTRICTOR	
	Adrenaline n=7	norAdrenaline n=7
Control	98.53 ± 0.07	98.38 ± 0.11
HA Constrictor	*** 96.59 ± 0.30	*** 96.25 ± 0.36
HA Phentolamine	98.37 ± 0.10	98.33 ± 0.05
HA Constrictor + Phentolamine	98.42 ± 0.09	98.36 ± 0.10
PV Constrictor	*** 97.63 ± 0.12	*** 97.26 ± 0.17
PV Phentolamine	98.40 ± 0.12	98.34 ± 0.08
PV Constrictor + Phentolamine	98.37 ± 0.08	98.32 ± 0.07

*** = significance < 0.005 using the Mann Whitney test.

Values are percentages ± standard errors of the means.

Appendix 29

To Show the Effect of Increases in Perfusion Pressure due to Infusions of Adrenaline or norAdrenaline on Combined Total Metabolite Output Concentration Expressed as a Percentage of Lignocaine Input Dose concentration in nM/ml.

TREATMENT	CONSTRICTOR	
	Adrenaline n=7	norAdrenaline n=7
Control	67.23 ± 3.27	67.61 ± 6.72
HA Constrictor	60.03 ± 2.86	64.00 ± 2.75
HA Phentolamine	70.91 ± 3.02	65.11 ± 2.55
HA Constrictor + Phentolamine	70.11 ± 3.91	67.93 ± 2.04
PV Constrictor	66.90 ± 3.63	64.91 ± 2.27
PV Phentolamine	70.14 ± 2.70	66.34 ± 4.84
PV Constrictor + Phentolamine	75.20 ± 5.08	66.41 ± 2.99

Values are percentages ± standard error of the means.

Appendix 30

The Effect of Increases in Perfusion Pressure due to Infusions of Adrenaline or norAdrenaline on Total 3-OH Lig Metabolite Output Expressed as a Percentage of Combined Total Output metabolite concentration in nM/ml.

TREATMENT	CONSTRICTOR	
	Adrenaline n=7	norAdrenaline n=7
Control	22.31 ± 1.87	21.89 ± 1.45
HA Constrictor	22.31 ± 1.41	21.74 ± 1.51
HA Phentolamine	21.63 ± 1.76	22.00 ± 1.63
HA Constrictor + Phentolamine	22.13 ± 1.52	21.51 ± 1.31
PV Constrictor	22.20 ± 1.16	21.81 ± 1.66
PV Phentolamine	21.34 ± 1.63	22.93 ± 1.51
PV Constrictor + Phentolamine	20.74 ± 1.56	21.93 ± 1.89

Values are percentages ± standard error of the means.

Appendix 31

The Effect of Increases in Perfusion Pressure due to Infusions of Adrenaline or norAdrenaline on Total 3-OH MEGX Output Expressed as a Percentage of Combined Total Output metabolite concentration in nM/ml.

TREATMENT	CONSTRICTOR	
	Adrenaline n=7	norAdrenaline n=7
Control	43.73 ± 1.56	42.16 ± 0.80
HA Constrictor	41.13 ± 0.56	40.40 ± 1.13
HA Phentolamine	42.90 ± 1.13	42.71 ± 1.00
HA Constrictor + Phentolamine	41.49 ± 0.85	42.57 ± 1.02
PV Constrictor	41.14 ± 0.84	42.59 ± 2.36
PV Phentolamine	40.74 ± 1.26	42.70 ± 1.46
PV Constrictor + Phentolamine	41.97 ± 1.10	43.06 ± 1.92

Values are percentages ± standard error of the means.

Appendix 32

The Effect of Increases in Perfusion Pressure due to Infusions of Adrenaline or norAdrenaline on Total De-ethylated (MEGX + GX) Metabolite Output Expressed as a Percentage of Combined Total Output metabolite concentration in nM/ml.

TREATMENT	CONSTRICTOR	
	Adrenaline n=7	norAdrenaline n=7
Control	31.61 ± 1.90	33.47 ± 1.43
HA Constrictor	27.26 ± 4.81	31.71 ± 1.89
HA Phentolamine	33.43 ± 1.81	32.70 ± 1.57
HA Constrictor + Phentolamine	34.19 ± 1.92	33.47 ± 1.33
PV Constrictor	33.23 ± 1.65	32.06 ± 2.09
PV Phentolamine	35.79 ± 2.26	31.66 ± 2.35
PV Constrictor + Phentolamine	34.97 ± 1.85	33.06 ± 1.52

Values are percentages ± standard error of the means.

Appendix 33

The Effect of Increases in Perfusion Pressure due to Infusions of Adrenaline or norAdrenaline on Total Glucuronide Conjugate Output Expressed as a Percentage of Combined Total Output metabolite concentration nM/ml.

TREATMENT	CONSTRICTOR	
	Adrenaline n=7	norAdrenaline n=7
Control	61.19 ± 1.53	58.00 ± 3.00
HA Constrictor	55.27 ± 1.84	55.97 ± 2.90
HA Phentolamine	62.75 ± 2.90	58.94 ± 3.70
HA Constrictor + Phentolamine	60.60 ± 2.30	56.82 ± 3.31
PV Constrictor	57.68 ± 3.46	59.87 ± 4.58
PV Phentolamine	66.24 ± 1.65	59.06 ± 4.06
PV Constrictor + Phentolamine	63.90 ± 2.80	55.74 ± 3.37

Values are percentages ± standard error of the means.

Appendix 34

The Effect of Increases in Perfusion Pressure due to Infusions of Adrenaline or norAdrenaline on Total Sulphate Conjugate Output Expressed as a Percentage of Combined Total Output metabolite concentration in nM/ml

TREATMENT	CONSTRICTOR	
	Adrenaline n=7	norAdrenaline n=7
Control	17.90 ± 2.33	21.38 ± 2.65
HA Constrictor	20.04 ± 2.58	21.28 ± 2.81
HA Phentolamine	19.56 ± 3.27	23.13 ± 3.42
HA Constrictor + Phentolamine	20.94 ± 2.28	20.94 ± 1.95
PV Constrictor	22.14 ± 3.54	18.00 ± 3.14
PV Phentolamine	15.89 ± 2.67	20.38 ± 2.19
PV Constrictor + Phentolamine	17.30 ± 3.28	22.84 ± 1.91

Values are percentages ± standard error of the means.

Appendix 35

The Effect of Increases in Perfusion Pressure by Adrenaline or norAdrenaline Infusions on Unconjugated Metabolite Output concentration Experssed as a Percentage of Combined Total Output Metabolite Concentration in nM/ml.

TREATMENT	CONSTRICTOR	
	Adrenaline n=7	norAdrenaline n=7
Control	18.86 ± 2.07	18.16 ± 4.46
HA Constrictor	19.56 ± 2.08	16.71 ± 3.34
HA Phentolamine	15.54 ± 1.81	15.53 ± 2.78
HA Constrictor + Phentolamine	16.30 ± 1.85	19.03 ± 3.48
PV Constrictor	16.74 ± 1.65	18.33 ± 4.28
PV Phentolamine	15.70 ± 2.07	18.13 ± 3.34
PV Constrictor + Phentolamine	16.16 ± 2.59	19.00 ± 3.96

Values are percentages ± standard error of the means.

Appendix 36

The Effect of Hydralazine, 1mg/kg, given Orally on Systolic Blood Pressure

TIME AFTER DOSE	PRESSURE mmHg ± s.e.m.	
	Control n=3	Hydralazine n=3
0	112 ± 3.0	121 ± 6.7
15	119 ± 4.3	120 ± 4.3
30	115 ± 9.3	113 ± 6.0
45	120 ± 5.9	* 95 ± 2.8
60	129 ± 4.5	** 81 ± 2.8
90	107 ± 4.6	* 90 ± 5.0
120	120 ± 4.9	129 ± 5.3
180	123 ± 4.7	118 ± 10.3

Values are means ± standard error of the mean.

Significance was assessed using the unrelated "t" test.

* $p < 0.05$

** $p < 0.01$

Appendix 37

The Effect of Hydralazine, 1mg/kg, given Orally on the Clearance of Lignocaine, 20mg/kg, Orally or 2.5mg/kg, Intravenously

	PLASMA CONCENTRATION ng/ml			
	ORAL LIGNOCAINE		I.V. LIGNOCAINE	
Time After Dose (mins)	Control n=3	Hydralazine Treated n=6	Control n=3	Hydralazine Treated n=6
5	703 ± 7.3	573 ± 21.6	1217 ± 105.0	1323 ± 100.0
10	1642 ± 143.3	1221 ± 75.7	606 ± 5.5	826 ± 37.0
15	1794 ± 10.4	1531 ± 29.2	497 ± 2.8	624 ± 7.9
20	1101 ± 96.5	1750 ± 26.2	427 ± 12.1	538 ± 17.3
30	800 ± 36.3	1211 ± 50.5	322 ± 9.7	420 ± 24.4
45	595 ± 75.9	865 ± 83.0	180 ± 31.5	216 ± 23.1
60	427 ± 12.1	690 ± 31.0	123 ± 15.4	155 ± 20.4
90	178 ± 9.1	405 ± 20.0	73 ± 2.8	100 ± 4.9
120	117 ± 30.9	280 ± 19.7	- -	- -
180	40 ± 8.3	160 ± 16.0	- -	- -

Values are means ± standard error of the mean.

Appendix 38

The Effect of Hydralazine, 1mg/kg, on the Plasma Clearance of Indocyanine Green

Time after dye Injection (mins)	PLASMA CONCENTRATION ug/ml	
	Control n=3	Treated n=3
1	0.275 ± 0.009	0.290 ± 0.005
3	0.192 ± 0.011	0.236 ± 0.005
5	0.145 ± 0.010	0.173 ± 0.004
7	0.114 ± 0.006	0.139 ± 0.002
10	0.081 ± 0.006	0.109 ± 0.002
15	0.047 ± 0.003	0.064 ± 0.001
20	0.036 ± 0	0.047 ± 0.002
25	0.031 ± 0.001	0.037 ± 0.001
30	0.027 ± 0.001	0.032 ± 0
45	0.024 ± 0	0.030 ± 0
60	0.020 ± 0.001	0.026 ± 0.001

Values are means ± standard error of the mean.

CHAPTER 6

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